VNIVERSITAT DE VALÈNCIA

TESIS DOCTORAL

Desarrollo de nuevas estrategias analíticas basadas en la espectrometría de masas para la determinación de contaminantes procedentes de la migración de materiales plásticos en alimentos

DEPARTAMENTO DE QUÍMICA ANALÍTICA

PROGRAMA DE DOCTORADO: Cód. 3158.

DOCTORADO EN TÉCNICAS EXPERIMENTALES EN QUÍMICA

LÍNEA DE INVESTIGACIÓN: SEGURIDAD ALIMENTARIA

YOVANA MARÍA SANCHIS BOIX

DIRECTORES:

Dr. Vicent Yusà Pelechá

Dra. Clara Coscollà Raga

Tutor: Dr. Agustín Pastor García

Valencia

Octubre 2019

Esta Tesis ha producido los artículos:

1. Analytical strategies for organic food packaging contaminants, 2017. Journal of Chromatography A 1490, 22-46 (Q1)
2. Target analysis of primary aromatic amines combined with a comprehensive screening of migrating substances in kitchen utensils by liquid chromatography-high resolution mass spectrometry, 2015.Talanta 138, 290-297 (Q1)
3. Comprehensive analysis of photoinitiators and primary aromatic amines in food contact materials using liquid chromatography High-Resolution Mass Spectrometry, 2019. Talanta 191, 109-118 (Q1)
4. Analysis of four Parabens and Bisphenols A, F, S in urine, using Dilute and Shoot and liquid chromatography coupled to mass spectrometry, 2019. Talanta 202, 42-50 (Q1)
5. Biomonitoring of bisphenols A, F, S and parabens in urine of breastfeeding mothers: exposure and risk assessment, 2019 (En revision).

AGRADECIMIENTOS

Gracias a todos y cada uno de los que estáis dedicando vuestro tiempo a la lectura de esta tesis, especialmente,

A mis directores de tesis: la Dra. Clara Coscollà, con la que me inicié en este largo camino de la tesis doctoral y el Dr. Vicent Yusà al que ha sido y es una fuente de energía, entusiasmo y rigor científico, gracias.

A Pedro Martí y Carmen Igualada por haber confiado en mi trabajo, a todos los compañeros que lo han hecho posible y en especial a Paco Moragues por su apoyo incondicional, a Carmen Forcada por entenderme en cada etapa, a Nuria, Maribel, Antonio y Pablo por sus consejos.

A mis padres por serlo todo, por esos buenos y malos momentos, por creer en mí. A mi hermana por estar a pesar de la distancia.

A Lluís y a Marc por la paciencia, el apoyo diario y sobre todo por "esas muchas horas robadas".

A mis padres,

A Lluís,

A Marc

Índice

TABLAS

FIGURAS

ABREVIATURAS

12 DMNa	1,2 Dimetilnaftaleno
14 DMNa	1,4 Dimetilnaftaleno
1-5 DAN	1-5 diaminodifenilmetano
16 DMNa	1,6 Dimetilnaftaleno
1mfLN	1-Metilfluoreno
1MNa	1- metilnaftaleno
2,4 Di-6-P	2,4 dibutil-6- nitrofenol
2,4,5 MTA	2,4,5 trimetilanilina
2-4 TD4	2,4 diaminotolueno
2-6 TDA	2,6 diaminotolueno
26DMnA	2,6 Dimetilnaftaleno
27 DiPNa	2,7 Disopropilnaftaleno
2-Eac	2- etilhexilacetato
2ITX	2- Isopropiltioxanona
2Mant	2- metilantraceno
2MNa	2- metilnaftaleno
2-NAPH	2- Naftilamina
3,5 M-O-M	3,5 Dimetil o- metiloxime
3-3’ DCD	3-3 Diclorobenzidina
4-4 MDA	4-4 Metilenodianilina.
4-4’ DPE	4-4’ Diaminodifenileter
4-4’ M (2Cl)	4-4’ metilen-bis-(2cloroanilina)
4-4’ MBM	4-4’ Metilene-bis-(2-metilanilina)
4-4’ TDA	4-4’ tiodianilina
4-ABF	4 Aminobifenilo
4-Cl-TOL	4- Cloro-o-toludina
4ITX	4- Isopropiltioxanona
4MBFN	4- Metilbenzofenona
5-N-O-TOL	5 Nitro-O-toludina
9Mant	9- metilantraceno

9-ODA	9 Octadecenamida
ABC	Ácidos abieticos
Ace	Acenafteno
Acy	Acenaftileno
IDA	Ingesta Diaria Admisible
Adv-800	Advastab 800
AG	Gas Auxiliar
ANL	Anilina.
Ant	Antraceno
APCI	Ionización a Presión Atmosférica
APEO	Nonilfenil Polietoxilado
BaA	Benzoantraceno
BADGE	2,2´-Bis(4-glicidiloxifenil)propano
BADGE· 2H2O	2,2´-Bis[4-(2,3-dihidroxipropoxi)fenil]propano
BADGE· 2HCl	2,2´-Bis[4-(3-cloro-2-hidroxipropoxi)fenil]propano
BADGE· H2O	2-[4-(2,3-Dihidroxipropiloxi)fenil]-2-[4-(glicidiloxi)fenil]propano
BADGE· HCl	2-[4-(3-Cloro-2-hidroxipropiloxi)feni]-2-[4-(glicidiloxi)fenil]propano
BADGE·H2O·HCl	2-[4-(3-Cloro-2-hidroxipropiloxi)fenil]-2-[4-(2,3dihidroxipropiloxi) fenil] propano
BaP	Dibenzoantraceno
bBf	Benzofluoroanteno
BBP	Benzylbutyl ftalato
BE	Biomonitoring equivalent
BFDGE	Bisfenol F diglicidiléter
BFN	Benzofenona
BghiP	Benzoperileno
BkF	Benzopireno
BMEL	Ministerio Federal Alemán de alimentos y agricultura
BNZ	bencidina
BP	Butilparabeno
BPA	Bisfenol A
BPF	Bisfenol F

BPS	Bisfenol S
BPs	Bisfenoles
CCD	Diseño Central Compuesto
CF-SPME	Microextracción en fase sólida con corriente de nitrógeno
Ch-81	Chimassorb 81
Chr	Criseno
COP	Contaminante Orgánico Persistente
CT	Temperatura del Capilar
Cys UV12	Ciasorb UV12
Cys UV24	Ciasorb UV24
Cys UV5411	Ciasorb UV5411
Cys UV9	Ciasorb UV9
Cyx-2246	Cyanox 2246
DAP	Dailil Ftalato
DBEP	Di (2- butoxietil) Ftalato
DBeP	DibenzilFtalato
DBP	Dibutiloftalato
DC	Corriente de Descarga
DCHP	DiciclohexilFtalato
DEHP	DietilhexilFtalato
DEP	Ftalato dietílico
DHP	DiheptilFtalato
DHXP	DihexilFtalato
DIBP	Di-iso-butil Ftalato
DiDP	Di- isodecil Ftalato
DIDP	Di-iso-decilglucósido Ftalato
DiHP	Di-n- heptyl Ftalato
DINP	Di-iso- nonil Ftalato
DIPrp	Diisopropil Ftalato
DI-SPME	Microextracción en Fase Sólida con Inmersión Directa
DMEP	Di (2-metoxietil) Ftalto
DMP	Dimetil Ftalato

DnBP	Ftalato de Di-n-butilo
DNOP	Dioctil Ftalato
DoE	Diseño de Experimentos
DPhP	Difenil Ftalato
DPP	Dipentil Ftalato
EDB	2Etil-4 DimetilaminoBenzoato
EE. UU.	Estados Unidos
EFSA	Autoridad Europea de Seguridad Alimentaria.
EI	impacto electrónico
EP	Etilparabeno
ESI	Ionización por Electrospray
EuPIA	Asociación Europea de Tintas de Impresión
FD	Frecuencia de detección.
FID	Detector con Ionización en modo Llama
Fln	Fluoreno
Flt	Fluoroanteno
FOSAs	perFluorOctanoSulfonAmidas
FOSEs	perFluorOctanoEtanol Sulfonamidas
FTOHs	Alcoholes FluoroTelomeros
FUSLE	Extracción Sólido Liquido con Ultrasonidos
GC	Cromatografía de Gases
GC-MS/MS	Cromatografía Gaseosa con detección Masas Masas
GPC	Cromatografía de Permeabilidad en Gel
GP-MSE	Microjeringa de Purga de Gas
HBM	Estudios de biomonitorización humana.
HDPE	Polietileno de Alta Densidad.
HE	1- Hexanol-2-Etil
HS-SPME	Micro extracción en fase Sólida con Espacio de Cabeza
HQ	Cociente de Riesgo
I-1010	Irganox 1010
I-1076	Irganox 1076
I-1081	Irganox 1081
I-1330	Irganox 1330

I-168	Irgafos 168
IcdP	Indenopireno
IDA	Ingesta Diaria Admisible
IDE	Ingesta Diaria Estimada
IDT	Ingesta Diaria Temporal
LAC	Lactona Cíclica
LAS	N2- dodecanoilarginina
LC- HRMS	Cromatografía Liquida de Alta Resolución con detector Masas
LC	Cromatografía Liquida
LC-MS/MS	Cromatografía Liquida con detección Masas.
LD	Limite de Detección
LDPE	Polietileno de Baja Densidad.
L-L	Extracción Liquido-Liquido
LLDPE	Polietileno Lineal de Baja Densidad.
LME	Limite de Migración Específica.
LMG	Límite de Migración Global.
LC	Limite de Cuantificación
MCA	Materiales en Contacto con Alimentos
MOAH	Mezclas Complejas de Hidrocarburos Insaturados
MOSH	Mezclas Complejas de Hidrocarburos Saturados
MP	MetilParabeno
m-PDA	FenilenDiAmina
MS/MS	Masas/Masas.
Na	Naftaleno
NIAS	Sustancias Añadidas de Forma No Intencionada
NOAELs	"*No observed adverse effect level",* dosis más alta, en la curva dosis respuesta, que no produce un efecto adverso observable.
NP	NonilFenol
o-AaT	o-AminoAzoTolueno
o-ANS	o-Ansidina
o-DANS	o- DiAnsidina
OP	OctilFenol

o-TOL	o- Toludina
o-TOLI	o- Tolidina
PA	PoliAmida.
PAAs	Aminas Aromáticas Primarias
p-ABZ	p- AminoBenzeno
PAEs	Ftalatos
PAH's	Hidrocarburos Aromáticos Policiclicos
p-ANL	p- CloroAnilina
PAPs	Esteres del Fosfato de Polifluoro Alquilo
PBDE's	Difenilos PoliBromados
PC	PoliCarbonato.
PCB's	Difenilos PoliClorados
p-CRS	p- CreSidina
PCP	Productos para el cuidado personal
PE	PoliEtileno.
PET	PoliEtilenTereftalato.
PFAs	. PoliperFluoroAlquil acidos
PFBA	Acido PerFloroButanoico
PFBS	Acido PerFluoroButanoSulfonico
PFCAs	PoliperFluoroalquil Acidos Carboxilados.
PFCs	Compuestos PerFluorados
PFDA	Acido PerFluoroDecanoico
PFDoA	Acido PerFluoroDodecanoico
PFDPA	Acido PerFluoroDecanoFosfonico
PFHpA	Acico PerFluoroHeptanoico
PFHxA	Acido PlerFluoroHexanoico
PFHxPA	Acido PerFluoroHexanoFosfonico
PFHxS	Acido PerFluoroHexanoSulfonico
PFNA	Acido PerFluoroNonanoico
PFOA	Acido PerFluoroOctanoico
PFOPA	Acido PerFluoroOctanoFosfonico
PFOS	PerfluoroOctano Sulfonato
PFPA	Acido PerFluoroPropanoico

PFPeA	Acido PerFluoroPentanoico
PFRS	Retardantes de llama fosforados.
PFSAs	PoliperFluoroalquil Acidos Sulfonados.
PFUnA	Acido PerFluoroUndecanoico
Phe	Fenantraceno
PLE	Extracción Líquida Presurizada
PP	PropilParabeno
PRP	PoliPropileno.
PS	PoliEstireno.
PVA	PoliVinilAcetato.
PVC	PoliClourodeVinilo.
Pyr	Pirreno
QuEChERS	Extracción en Fase Sólida Dispersiva (Quick, Easy, Cheap, Effective, Rugged & Safe)
QTOF	Analizador de tipo cuadrupolo (Q) y tiempo de vuelo (TOF).
RASFF	Sistema de Alerta Rápido para Alimentos y Piensos
Ref	Referencias
SGP	Presión del Gas Envolvente.
SIM	Monitorización Selectiva de Iones.
S-L	Extracción Solido Liquido
SV	Voltaje de Espray
SPE	Extracción en Fase Sólida
SPME	Micro extracción en Fase Sólida
TDMM	Tensioactivo de Etoxilato
TNPP	Tris (NonilFenil) Fosfato
TNV 234	Tinuvin 234
TNV 326	Tinuvin 326
TNV 327	Tinuvin 327
TNV 328	Tinuvin 328
TOF	Tiempo de vuelo.
UE	Unión Europea
UHPLC	Cromatografía Liquida de ultra Alta Presión

UHPLC-HRMS	Cromatografía Líquida de ultra Ata Presión acoplada a espectrometría de masas de alta resolución.
UV-400	Uvinul 400
Uv-OB	Uvitex OB
VT	Temperatura de Vaporización

ABSTRACT/RESUMEN

La presencia de contaminantes en los alimentos, y consecuentemente la exposición humana a los mismos, es uno de los problemas más relevantes en el ámbito de la seguridad alimentaria y la salud pública.

En el campo alimentario, el uso de envases resulta imprescindible para proteger los alimentos y evitar su degradación. Actualmente, la migración de los contaminantes desde los materiales que conforman el envase a los alimentos es una de las mayores preocupaciones de las autoridades sanitarias. El Reglamento (CE) 1935/2004 regula los requisitos que deben cumplir todos los materiales y objetos (madera, vidrio, metal, cerámica, plástico, etc.) destinados a entrar en contacto directo o indirecto con los alimentos, así como su procedimiento de autorización, etiquetado y trazabilidad. El plástico es el material más utilizado en la industria alimentaria y son numerosas las sustancias asociadas a éste. El Reglamento (UE) 10/2011 describe la "Lista de la Unión" con más de 900 sustancias autorizadas en la fabricación de plásticos destinados a alimentos, con límites de migración específica establecidos para muchas de ellas.

Esta tesis se ha centrado en los grupos de contaminantes orgánicos más relevantes y prioritarios, sobre los que en los últimos años ha habido mayor interés en su determinación, debido a los posibles problemas de salud que pueden causar a la población, especialmente en grupos vulnerables como los niños o las mujeres embarazadas. Estas sustancias son: aminas aromáticas primarias, bisfenoles A, F y S, bisfenol A éter diglicidil y compuestos relacionados, fotoiniciadores, compuestos perfluorados, ftalatos y sustancias añadidas de forma no intencionada.

Esta Tesis Doctoral tiene como objetivo principal el desarrollo de nuevas metodologías analíticas adecuadas para el análisis de contaminantes orgánicos procedentes de los materiales destinados a entrar en contacto con los alimentos, mediante la utilización de la cromatografía líquida acoplada a la espectrometría de masas. Además, se ha utilizado la biomonitorización humana para evaluar la exposición y el riesgo de una población de mujeres lactantes a distintos contaminantes procedentes de envases plásticos.

En la introducción de la tesis se ha realizado una revisión crítica del estado actual de las metodologías analíticas para la determinación de contaminantes derivados de los materiales. Los resultados de la tesis constan de cuatro capítulos, los tres primeros

orientados al desarrollo de nuevas estrategias analíticas para la determinación de los distintos grupos de contaminantes orgánicos procedentes de envases o utensilios en contacto con los alimentos, y el cuarto centrado en la evaluación de la exposición interna y el riesgo en una población de madres lactantes.

En el primer capítulo se ha desarrollado una metodología analítica que combina el análisis cuantitativo de aminas aromáticas con un cribado amplio de contaminantes sospechosos, mediante el UHPLC-HRMS, aplicando el método optimizado y validado a 10 muestras de utensilios de poliamida.

En el capítulo 2 se ha desarrollado una metodología analítica para la determinación cuantitativa de aminas aromáticas primarias y fotoiniciadores, y el análisis retrospectivo de contaminantes en diferentes tipos de envases alimentarios, mediante el uso del UHPLC-HRMS. Para ello, se ha desarrollado una base de datos de 87 compuestos y el método analítico validado se ha aplicado a 18 muestras procedentes de distintos envases.

En el capítulo 3 se ha diseñado una estrategia analítica para la determinación cuantitativa de bisfenoles (BPA, BPF y BPS) y parabenos (MP, EP, PP y BP) en orinas, mediante LC-MS/MS.

En el cuarto capítulo se aplicó la metodología desarrollada en el capítulo 3 a un estudio de biomonitorización de bisfenoles y parabenos en una población de madres lactantes. Se analizaron un total de 103 muestras de orina. Mediante el uso de herramientas estadísticas se han estudiado los factores de influencia en los niveles de parabenos y bisfenoles. Se calcularon las ingestas diarias estimadas (IDE), los cocientes de riesgo (HQ) y márgenes de seguridad en función de los valores de referencia disponibles para cada compuesto.

Esta serie de trabajos, tanto las metodologías analíticas desarrolladas como el estudio de evaluación del riesgo a la exposición de bisfenoles y parabenos, pueden ayudar para posibles normativas futuras en el uso y restricción de contaminantes orgánicos en los envases y/o utensilios destinados a alimentos.

1. Introducción

1.1. Materiales en contacto con los alimentos (MCA)

1.1.1. MCA y Salud Pública

La calidad y seguridad de los alimentos es una de las preocupaciones más importantes en el ámbito de la salud pública. Las posibilidades de contaminación de un alimento comienzan ya en la obtención y tratamiento de las materias primas (producción primaria) y continúan a lo largo de la cadena alimentaria hasta el momento de su consumo (WHO 2002) (figura 1). Contaminantes como los plaguicidas pueden estar presentes en el alimento desde la recolección de las cosechas (producción primaria), mientras que sustancias como los hidrocarburos policíclicos aromáticos pueden formarse en el tratamiento culinario.

Entre los contaminantes alimentarios destacan los procedentes de los materiales en contacto con los alimentos (MCA), que son todos aquellos artículos y/o materiales destinados a entrar en contacto con los mismos, ya sean materiales de envasado, máquinas de procesado o utensilios de cocina. Dentro de los materiales en contacto con los alimentos encontramos papel, cartón, madera, metal, vidrio, cerámica y plástico.

Estos contaminantes se incorporan, en general, en las últimas etapas de la cadena, migrando fundamentalmente desde los envases durante la comercialización de los alimentos o desde los utensilios durante el cocinado en el hogar (WHO 2019).

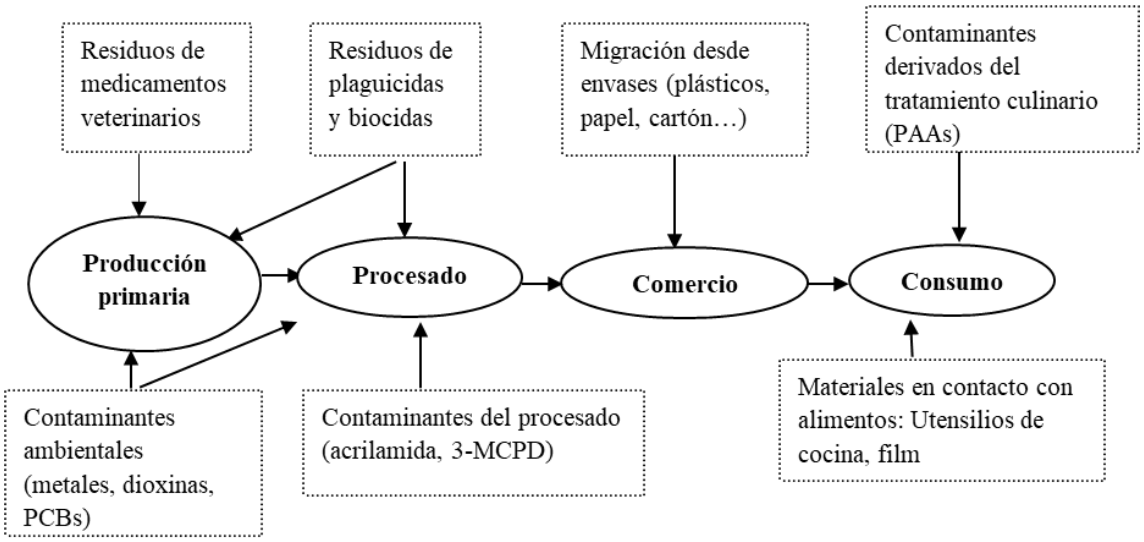


Figura 1. Ejemplos de contaminantes en las distintas etapas de la cadena alimentaria

El uso de los envases resulta, en muchas ocasiones, imprescindible actualmente para proteger al alimento frente a fuentes externas de contaminación y para evitar el deterioro

o la degradación del mismo. Actualmente, una de las mayores preocupaciones tanto de la población, como de las autoridades competentes y de la industria alimentaria es la migración de los contaminantes desde los materiales que constituyen el envase (MCA) a los alimentos. En este contexto, la vigilancia y control por parte de las autoridades públicas se ha establecido mediante normativas nacionales e internacionales aplicables a todos los materiales que entran en contacto directo con los alimentos. En el ámbito europeo, el Reglamento 1935/2004/EC es el que regula los materiales y objetos destinados a entrar en contacto con los alimentos y trata de garantizar un alto nivel de protección de la salud en este ámbito. Esta normativa engloba los distintos materiales, tales como cerámicas, corcho, caucho, vidrio, plástico, metal, papel, silicona, lana, etc.

El plástico es el MCA más utilizado debido a que presenta ventajas como su durabilidad, su flexibilidad y su bajo coste (Heidbreder et al 2019). La producción del plástico mundial ha aumentado en las últimas décadas, alcanzando 380 millones de toneladas en el año 2015, de las cuales el 40% se utilizó para el envasado (Geyer et al 2017, PlasticsEurope 2016). Es importante señalar que aproximadamente el 60% de todo el envasado plástico mundial se destina a alimentos y bebidas (PlasticsEurope 2018).

En el ámbito de la Unión Europea, el consumo de plástico ha ido incrementándose, estimándose un consumo de 46.3 millones de toneladas en 2013 y 49 millones de toneladas en 2015 (Frölich 2014; PlasticsEurope 2016). España fue el cuarto país con mayor demanda de plásticos en 2015, consumiendo el 7.7 % del total europeo (Statista 2018). Cabe señalar, que la industria del envasado es el sector con mayor demanda de plástico (40%) en Europa (PlasticsEurope 2018).

Actualmente, el Reglamento 10/2011 es el que regula en la Unión Europea los materiales y objetos plásticos destinados a entrar en contacto con los alimentos. Esta norma, en su Anexo I, describe la "Lista de la Unión", que es una lista positiva de monómeros y aditivos autorizados para utilizar en la fabricación de plásticos destinados a entrar en contacto con los alimentos.

Son numerosas las sustancias asociadas a los materiales plásticos en contacto con los alimentos. Groh et al. (2019) presentan una lista de más de 900 compuestos entre los que se encuentran ftalatos, aminas o parabenos. En general, las sustancias añadidas no intencionadamente (NIAS) (Vera et al. 2018; Nerín et al. 2013), las aminas aromáticas primarias (PAAs) (Campanella et al. 2015; Favaro Perez et al. 2019), los fotoiniciadores (Dawidowick et al. 2019; Gallart-Ayala et al. 2011), bisfenoles A, F y S (BPA, BPF, BPS)

(Zhou et al. 2019; Park et al. 2018), los compuestos perfluorados (PFCs) (Zafeiraki et al. 2014) y los ftalatos (García et al. 2019; Fierens et al. 2012) son los contaminantes más frecuentemente descritos en la literatura que migran desde los MCA plásticos hacia los alimentos (Sanchis et al. 2017).

La población está expuesta a los contaminantes procedentes de los MCA fundamentalmente a través de la dieta. Gallart-Ayala et al. (2013) señaló que, determinados compuestos como los bisfenoles, derivados epóxidos y ftalatos, están presentes en alimentos enlatados, pescado, carne y zumos. La presencia de estos contaminantes en los alimentos y consecuentemente la exposición humana a los mismos, es uno de los problemas más relevantes en el ámbito de la salud pública (WHO 2019). La exposición repetida durante largos periodos de tiempo a estas sustancias puede tener efectos tóxicos importantes, con los consiguientes problemas de salud para la población general, y más concretamente para grupos de población vulnerables como son las mujeres embarazadas, las madres lactantes y la población infantil (Siracusa et al. 2018). Huang et al. (2017) describió la presencia de BPA en la orina de la población adulta en 30 países distintos, Lee et al. (2017) detectó bisfenoles (BPA) en orina de mujeres embarazadas. También se ha detectado BPA y parabenos en la leche materna (Dualde et al 2019; Schlumpf et al 2010). Arbuckle et al. (2014) detectó niveles de ftalatos en la orina de mujeres. Yusà et al. (2015) recopiló los métodos analíticos para la determinación de pesticidas en orina y sangre. La presencia de estos contaminantes en distintos fluidos biológicos confirma su presencia en el cuerpo humano y de ahí la importancia de investigar estas sustancias y su migración desde los MCA a los alimentos, y la consecuente exposición y riesgo para los humanos.

1.1.2. Polímeros

Los materiales plásticos son actualmente los más utilizados para el envasado y el contacto de alimentos. Este es el material en el que se va a centrar la presente tesis.

De acuerdo con el Reglamento (UE) nº 10/2011, el plástico se define como “polímero al que pueden haberse añadido aditivos, y que es capaz de funcionar como principal componente estructural de materiales y objetos finales”. Los polímeros son macromoléculas formadas por moléculas de más bajo peso molecular, llamadas monómeros, unidas por el proceso de polimerización (Demaid et al. 1996). En la formación de los plásticos intervienen además de los polímeros otros compuestos que se

adicionan intencionadamente como coadyuvantes del proceso de fabricación, destinados a conseguir determinados efectos en las propiedades técnicas del producto, aditivos como plastificantes, colorantes, retardantes de llama, estabilizantes, lubricantes… (Eyerer 2010).

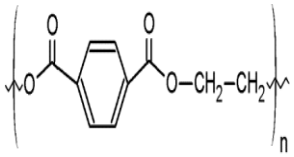
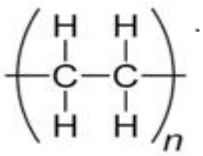
Los plásticos se pueden clasificar de diversas maneras, atendiendo a su composición química (homopolímero y heteropolímero), estructura de las cadenas (lineal, ramificada), origen (natural, sintético), estructura molecular (cristalino, amorfo). Conforme su comportamiento frente al calor, se dividen en tres grandes grupos:

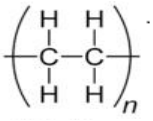
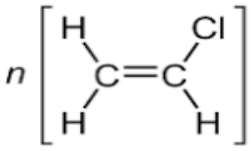
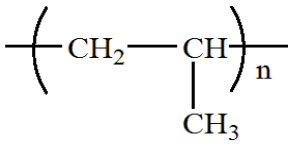
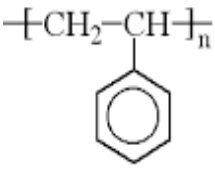
- **Termoplásticos:** Material polimérico unido por fuerzas de Van der Waals que a altas temperaturas puede fundirse, permitiendo luego darle diversas formas. Se derrite cuando se calienta y se endurece cuando se enfría. Esta propiedad es de gran ayuda para el reciclaje de plásticos, ya que después de calentarse y moldearse pueden recalentarse y formar otros objetos. Sin embargo, hay que tener en cuenta que, si se funden y se moldean varias veces, sus propiedades físicas cambian gradualmente disminuyendo su posibilidad de reutilización. Como ejemplos de estos materiales tenemos el polietileno (PE), poliestireno (PS), poliamida (PA), polietilentereftalato (PET) , policloruro de vinilo (PVC) y policarbonato (PC) (Eyerer 2010, Anzano et al. 2008)
- **Termoestables:** hacen referencia al conjunto de materiales formados por polímeros unidos mediante enlaces químicos adquiriendo una estructura final altamente reticulada. La estructura altamente reticulada que poseen los materiales termoestables es la responsable directa de las altas resistencias mecánicas y físicas que presentan dichos materiales comparados con los materiales termoplásticos y elastómeros. Por contra dicha estructura altamente reticulada es la que aporta una baja elasticidad, proporcionando a dichos materiales su característica fragilidad. Cómo ejemplos de estos materiales tenemos las resinas epoxi, siliconas y resinas poliéster (Eyerer 2010, Demaid et al. 1996).
- **Elastómeros:** son sustancias poliméricas que poseen la particularidad de moldearse en gran medida sin que lleguen a la zona de deformación plástica. Los elastómeros están unidos formando grandes cadenas, las cuales son altamente flexibles, desordenadas y entrelazadas. Cuando son

estirados, las moléculas son llevadas a una alineación y con frecuencia toman el aspecto de una distribución cristalina, pero cuando se las deja de tensionar retornan espontáneamente a su desorden natural, un estado en que las moléculas están enredadas. Esta forma de volver a su estado natural de desorden distingue a los elastómeros de los polímeros termoestables, los cuales son duros y frágiles. Como ejemplos de estos materiales el poliisopreno o caucho natural, el polibutadieno, el poliisobutileno y los poliuretanos (Demaid et al. 1996, Eyerer 2010)

Los termoplásticos son los más utilizados en la industria alimentaria para el envasado de los alimentos, así como para el recubrimiento en los materiales en contacto con los alimentos. De éstos, los materiales más habituales están detallados en la tabla.

Tabla 1. Características de los principales termoplásticos

Clasificación	Estructura	Propiedades Fisicoquímicas	Ejemplos	Referencias
PET 		Ligero, rígido o semirrígido, naturalmente transparente e incoloro, el PET es una excelente barrera contra la penetración de la humedad y las sustancias gaseosas. Resistente a los golpes, es uno de los plásticos más adecuado para reciclar.	Se utiliza para producir botellas, bolsas, fibras sintéticas para la ropa.	(Wypych 2012)
HDPE 	Linear con pocas ramificaciones cortas 	Es translúcido, sólida y fácil de trabajar, resistente a los impactos y no tóxico.	Se utiliza para producir botellas, contenedores, cestas, contenedores de transporte	(Wypych 2012)

Clasificación	Estructura	Propiedades Fisicoquímicas	Ejemplos	Referencias
LDPE 	Ramificada con muchas ramificaciones largas. 	Se puede encontrar de translúcido a transparente. Adecuado para el contacto con alimentos, es el plástico más ligero y sensible al calor	Se utiliza para producir embalajes, bolsas, bolsas, cubiertas de cables, contenedores, tuberías y juguetes.	(Wypych 2012)
PVC 		Resistente al desgaste, a los agentes químicos y atmosféricos, y al fuego	Se utiliza para producir envases y productos de papel, envases de alimentos, tarjetas de crédito, muebles, ropa y juguetes.	(Wypych 2012)
PRP 		Transparente, ligero y sólido, es un material plástico que se presta para un doble uso, tanto como plástico como fibra. Fácil de colorear, no absorbe el agua.	Se utiliza para producir fibras textiles, juntas, contenedores de transporte, muebles, contenedores de alimentos	(Wypych 2012)
PS 		Transparente, duro, inflamable e inerte frente a agentes corrosivos	Material de embalaje (para alimentos), recipientes, cajas, lámparas, artículos desechables, juguetes.	(Wypych 2012)

PET: polietilentereftalato, HDPE: polietileno de alta densidad, LDPE: polietileno de baja densidad, PVC: policloruro de vinilo, PRP: polipropileno, PS: poliestireno. Símbolo Δ : código del etiquetado.

Polietileno tereftalato (PET)

Es un poliéster formado por la reacción del ácido Tereftálico con el etileno glicol. Debido su estabilidad a un amplio rango de temperaturas (temperaturas negativas y de cocción), elasticidad, resistencia química y ligereza, lo convierten en un excelente material para la industria alimentaria. Ha sido y es ampliamente utilizado para el envasado de agua, zumos, bebidas carbonatadas y aceites (Wypych 2012).

Polietileno (PE)

Polímero obtenido mediante la polimerización de alquenos, debido a la polimerización por adición del monómero etileno (Nerín 2016, Eyerer 2010). Atendiendo a su densidad existen dos tipos de polietileno, el polietileno de baja densidad (LDPE) y el polietileno

de alta densidad (HDPE). El LDPE es uno de los polímeros más usados en la industria alimentaria, por su versatilidad y precio, así como por sus propiedades técnicas: flexibilidad y buena resistencia térmica y es una barrera eficaz al agua y al vapor, fácilmente utilizable cómo recubrimiento (Anzano, Lasheras et al. 2008). Ejemplos de materiales compuestos por este tipo de polietileno son: botellas para agua de consumo, botellas compresibles para pulverizar fármacos, envase alimentario, laminaciones, película para forro, película encogible y estirable, aislante para cables y conductores, película para invernadero, tubería de riego y sistemas de irrigación (Alnaimi et al. 2007, Wypych 2012).

Existe también el polietileno linear de baja densidad (LLDPE) que es un copolímero con densidad similar al LDPE pero obtenido a presiones y temperaturas bajas por un proceso catalítico formando una cadena polimérica con un gran número de ramificaciones de pequeña extensión (Alnaimi et al. 2007). Como ejemplos de este tipo de material tenemos bolsas para pañal, costales para productos a granel, costales de uso pesado, bolsa de basura, películas estirables, geomembranas y película para envase.

Por último, el HDPE (polietileno de alta densidad) posee mayor densidad y grado de cristalinidad que el LDPE debido a la menor extensión de sus ramificaciones. Se obtiene por un proceso de polimerización similar al del LLDPE y posee gran resistencia tanto física como química lo que le permite hacer un buen efecto barrera y además tiene el punto de fusión muy elevado. Como ejemplos de productos de este tipo de material están bolsas para mercancía, bolsas para basura, botellas para leche y yogurt, cajas para transporte de botellas, envases para productos químicos, envases para jardinería, detergentes y limpiadores, frascos para productos cosméticos y capilares, recubrimientos de sobres para correo, sacos para comestibles, aislante de cable y alambre, contenedores de gasolina, entre otros (Papkov 1982, Wypych 2012).

Polipropileno (PRP)

Polímero muy utilizado en la industria alimentaria por su baja densidad y su alto punto de fusión (160º), lo que permite la fabricación de materiales que se van a someter a lavavajillas o microondas. Tiene una buena resistencia química y física (Anzano et al. 2008, Eyerer 2010, Wypych 2012). Como ejemplos de productos están los envases alimentarios aptos para microondas y envases para transporte de alimentos.

Poliestireno (PS)

El poliestireno es un polímero con buena resistencia a la fatiga, buen aislante y de color transparente por lo que es de gran uso en los materiales en contacto con alimentos tanto en envases como en utensilios de cocina. Existe también el poliestireno de alto impacto, que es un copolímero que resulta de la adición de butadieno aumentando su resistencia al impacto, y el poliestireno expandido y extruido que se caracteriza por tener gas en su estructura y por la consecuente baja densidad, generalmente utilizado en productos frágiles y cómo aislante (Wypych 2012).

1.1.3. Interacciones material-alimento

Los alimentos constituyen un sistema complejo, el cual está sujeto a una gran variedad de procesos químicos. Por esta razón el alimento no es un medio estable, sino que interacciona con su entorno, constituido en muchos casos por los materiales con los que está en contacto. La interacción entre el entorno y el alimento comprende tres tipos de fenómenos de transporte de materia: permeación, sorción y migración (figura 2). (Campos 2017).

a) La **permeación** es un proceso de transporte de materia del entorno al alimento y viceversa. Las sustancias implicadas en este proceso son fundamentalmente gases o aromas. La fuerza impulsora de este proceso es una diferencia en el gradiente de concentración de la sustancia.

b) La **sorción** es la transferencia de materia desde el alimento o el entorno externo hacia el material en contacto con el alimento. De esta manera se distinguen dos procesos de sorción: a) La adsorción, que es un proceso por el que átomos, iones o moléculas de gases, líquidos o sólidos disueltos son atrapados o retenidos en una superficie, la cual tiene lugar entre la superficie del envase alimentario y el entorno (ambiente externo), y b) La absorción en la cual la sustancia migrante se adhiere desde la matriz alimentaria al envase aumentando su volumen. La absorción es un problema relacionado con una pérdida de calidad organoléptica, ya que sustancias tales como aromas presentes en el alimento, pueden quedar retenidas en el material de envasado.

c) El último fenómeno de transporte, en el que se centra la tesis corresponde a la **migración**, que se define como la transferencia de materia desde el material en

contacto con alimento hacia el alimento. Este proceso es muy irregular y depende de distintos factores como la temperatura, el tiempo, la porosidad, etc (Arvanitoyannis et al. 2004). La migración conlleva peligros toxicológicos debido a la transferencia de determinados compuestos, como aminas, fenoles, fotoiniciadores, que se discutirán más adelante.

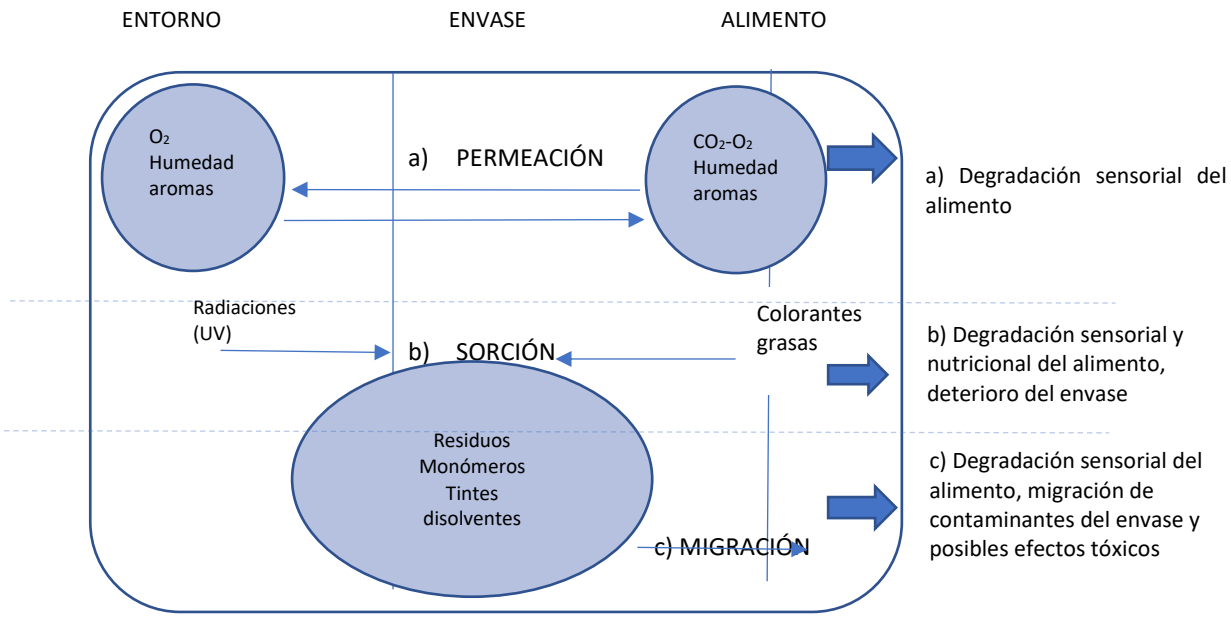


Figura 2. Fenómenos de transporte entre el envase, el alimento y el entorno

1.1.4. Migración desde materiales plásticos

El proceso de migración ocurre en tres etapas interrelacionadas, las cuales se detallan a continuación.

1. La primera de ellas es la difusión del migrante en la matriz polimérica. Este es un proceso en el cual los aditivos u otras sustancias capaces de migrar (monómeros, productos de transformación), experimentan un movimiento browniano dentro de la matriz polimérica obedeciendo a las leyes de difusión de materia de Fick (Anzano et al. 2008).
2. La segunda etapa de la migración se produce en la interfase entre el alimento y el envase; en este proceso el migrante se solubiliza en el alimento y pasa a éste. La cantidad de sustancia capaz de migrar depende en gran medida de la solubilidad

del compuesto migrante en el alimento, ya que una gran solubilidad del migrante implica una mejor solubilización y mayor difusión.

3. La tercera etapa del proceso de difusión corresponde a la dispersión del compuesto migrante en el alimento. En el caso de que el alimento sea un sólido o un líquido muy viscoso el proceso de migración obedece las leyes de transferencia de materia de Fick, mientras que en alimentos líquidos poco viscosos, el migrante se encuentra, generalmente, en una concentración bastante homogénea en todo el producto, y debido a la baja resistencia que ofrece el alimento, al ser poco o nada viscoso, el migrante ve favorecida su difusión (Navia et al. 2014, Nerín 2016)

En el proceso de migración influyen, además, varios factores:

- La concentración de migrante, ya que la migración de un componente es un proceso directamente proporcional a la concentración de este en el alimento, y por lo tanto cuanto mayor es la diferencia de concentraciones mayor es la cantidad de migrante que se transfiere al alimento.
- Las condiciones de temperatura, ya que generalmente a mayor temperatura mayor es la migración.
- El tiempo, puesto que un mayor tiempo de contacto favorece el proceso de migración.
- La superficie del material de envasado, ya que a mayor superficie hay más área de contacto entre el alimento y el envase, por tanto, se incrementa la migración.
- Las características químicas del migrante (polaridad, peso molecular…).
- Las vibraciones mecánicas en el material de envasado que facilitan el proceso de migración.

Migración global y específica

Para caracterizar el fenómeno de la migración, se utilizan los conceptos de migración global, que se refiere a la suma de todos los componentes del envase que se transfieren al alimento, y por otra de migración específica, que representa la cantidad de una sustancia concreta que se transfiere al alimento bajo ciertas condiciones. Ambas migraciones están sujetas a normativas y se evalúan a través de simulantes.

Un simulante es un producto que imita el comportamiento de un alimento o grupo de alimentos. Dada la complejidad de los productos alimenticios y la variedad de condiciones que surgen del contacto con los plásticos, se han establecido oficialmente diferentes simulantes que se pueden utilizar en la determinación de migración en alimentos (ver tabla 2).

Estos simulantes representan al alimento con el que va a entrar en contacto el material, y sobre ellos se realiza el ensayo de migración en las condiciones de temperatura y tiempo que vienen reguladas por el Reglamento 10/2011 (tablas 3 y 4). Dicho ensayo, se realiza considerando las peores condiciones en las que el alimento puede entrar en contacto con el material.

Tabla 2. Simulantes alimentarios según el Reglamento 10/2011

Simulante/Composición	**Tipo Alimento**	**Especificaciones del alimento**
A / Etanol 10%(v/v)	Alimentos con carácter hidrofílico capaces de extraer sustancias hidrofílicas	-
B/Ácido acético 3%(v/v)		pH < 4.5
C/ Etanol 20%(v/v)		Alimentos con contenido de alcohol hasta un 20% y para alimentos con cantidad importante de ingredientes orgánicos que lo hagan lipofílico
D1/ Etanol 50%(v/v)	Alimentos con carácter lipofílico y capaces de extraer sustancias lipofílicas	Alimentos con grado alcohólico superior al 20% y para emulsiones acuosas de aceite.
D2(*) / Aceite vegetal		Alimentos con grasas libres en la superficie
E / Poli(óxido de 2,6- difenil-p-Fenileno)	Alimentos secos	-

(*) En alimentos descritos como "Bebidas-Diversos: alcohol etílico sin desnaturalizar"), el aceite vegetal se sustituye por etanol 95%. Según Reglamento 10/2011.

Tabla 3. Tiempos de contacto para materiales destinados al contacto con alimentos

Tiempo de contacto	**Duración del ensayo**
t ≤ 5 min	5 min
5 min < t ≤ 0.5h	0.5 h
0.5 h < t ≤ 1h	1h
1h < t ≤ 2h	2h
2h < t ≤ 6h	6 h
6h < t ≤ 24h	24 h

Tiempo de contacto	Duración del ensayo
1 día < t ≤ 3 días	3 días
3 días <t ≤ 30 días	10dias
30 días	Ver las condiciones específicas

Tabla 4. Temperatura de ensayo según el uso de materiales destinados al contacto con alimentos

Temperatura de uso del material	Temperatura del ensayo
T ≤5ºC	5 ºC
5 ºC < T≤ 20ºC	20 ºC
20º C < t ≤ 40º C	40 ºC
40º C < t ≤ 70º C	70 ºC
70º C < t ≤ 100º C	100º C o temperatura de reflujo
100º C < t ≤ 121º C	121º C
121º C < t ≤ 130º C	130 ºC
130º C < t ≤ 150º C	150 ºC
150º C < t ≤ 175º C	175º C
>175 ºC	Ver las condiciones específicas

a) Migración global

- En el Reglamento 10/2011 el límite de migración global (LMG) viene definido como la cantidad máxima permitida de sustancias no volátiles liberada desde un material u objeto en simulantes alimentarios. Los materiales y objetos plásticos en contacto con los alimentos no cederán sus constituyentes a los simulantes alimentarios en cantidades que superen los 10 miligramos de constituyentes liberados por decímetro cuadrado de superficie de contacto (mg/dm^2).
- Para los envases de productos específicos para niños y lactantes se establece en el reglamento que los materiales no cederán a los simulantes

alimentarios cantidades que superen 60 miligramos de constituyentes liberados por kilogramos de simulante alimentario.

- La migración global se determina por gravimetría y dependiendo de la matriz el simulante a utilizar será graso o acuoso. En el caso de simulante acuoso, pasado el tiempo de contacto, se recoge el simulante, se evapora lentamente hasta sequedad y se determina la masa del residuo seco. Conociendo el área de la superficie del material y la masa del residuo seco es posible calcular la migración global.
- En el caso de que se utilice un simulante graso, el método es más complejo ya que no se puede evaporar el simulante, por lo que hay que conocer la masa del simulante antes y después del ensayo. Además, el simulante graso es parcialmente absorbido por el material plástico durante el ensayo, por lo que es necesario extraerlo y determinarlo posteriormente por gravimetría.

b) Migración específica

- El límite de migración específica (LME) se aplica a grupos de sustancias o sustancias individuales fijando la cantidad máxima que puede ser cedida al alimento o simulante; se expresa en mg de sustancia por kg de alimento. Se establece en base a estudios de ingesta diaria tolerable, partiendo del supuesto de que una persona de 60 kg consume 1 kg de alimento al día y que ese alimento contiene el nivel máximo permitido de esa sustancia. En el caso en el que no se establezca un límite de migración específica para determinadas sustancias, se aplicará el límite genérico de 60 mg/kg.
- Para la determinación analítica del LME se recurre a simulantes alimentarios. El tipo de simulante a utilizar viene estipulado en el Reglamento 10/2011 tal como se indica en la tabla 2.

1.1.5. Migración específica: contaminantes prioritarios

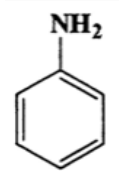
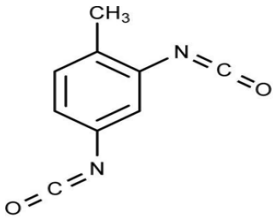
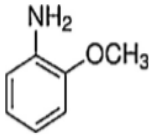
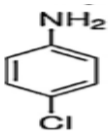
Como se ha señalado, existen muchos compuestos que pueden migrar de los materiales a los alimentos. Esta tesis, se ha centrado en las familias de compuestos orgánicos más relevantes y prioritarios, sobre los que en los últimos años ha habido mayor interés en su análisis debido a los posibles problemas de salud que pueden causar en la población,

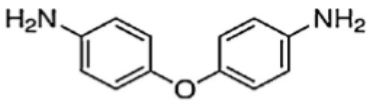
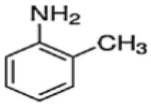
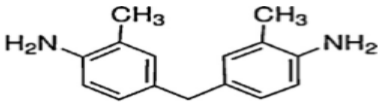
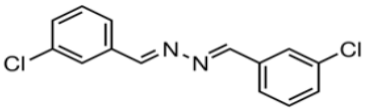
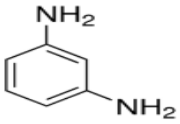
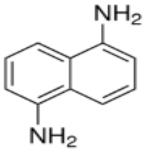
especialmente en niños. Estas sustancias son: aminas aromáticas primarias, bisfenol A, bisfenol A diglicidil éter y compuestos relacionados, fotoiniciadores, compuestos perfluorados, ftalatos y sustancias añadidas de forma no intencionada (Bach et al. 2012, Ballesteros et al. 2017, De Toni et al. 2017).

Aminas aromáticas primarias (PAAs)

Las aminas aromáticas primarias son sustancias muy reactivas por lo que se emplean en la fabricación de múltiples productos tales como tintas, textiles, poliuretanos, etc. Los poliuretanos utilizados en muchos casos como adhesivos en el proceso de curado de materiales aislantes para neveras o como conglomerantes de envases multicapa (fiambreras), pueden no reaccionar de forma completa (hidrólisis de isocianatos aromáticos) liberando aminas aromáticas primarias (Sanchis et al. 2015). También se forman por degradación de los grupos azoicos utilizados como colorantes en utensilios de cocina de nylon y otros materiales plásticos. Brede et al. (2003) identificaron los utensilios de poliamida como una fuente común de PAAs. En la Tabla 5 se encuentran las PAAs detectadas en MCA y los rangos de concentraciones halladas.

Tabla 5. Niveles, procedencia y estructura de PAAs

Aminas	Estructura	Procedencia	Niveles (µg/kg)	Referencia
ANL		Láminas de plástico y utensilios de cocina de poliamida	2.5-284	(Sanchis et al. 2015)
2,4 TDA		utensilios de cocina de poliamida	2.5-12	(Sanchis et al. 2015)
o-ANS		Láminas de plástico	< 2.5	(Sanchis et al. 2015)
p-clor		Láminas de plástico	<2.5	(Wypych 2012, Mattarozzi et al. 2013)

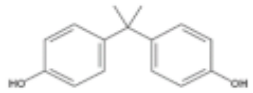
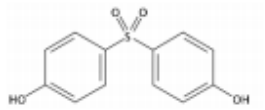
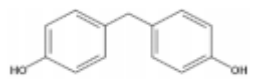
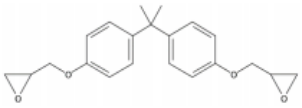
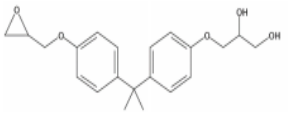
Aminas	Estructura	Procedencia	Niveles (µg/kg)	Referencia
4,4' DPE		utensilios de cocina de poliamida	<2.5	(Sanchis et al. 2015)
o-Tol		Láminas de plástico	<2.5	(Wypych 2012, Mattarozzi et al. 2013)
4,4' MDA		utensilios de cocina de poliamida	2-19.8	(Sanchis et al. 2015)
3,3' DCB		Láminas de plástico	2.7-49	(Wypych 2012, Mattarozzi et al. 2013)
1,3 m-PDA		utensilios de cocina de poliamida	2	(Sanchis et al. 2015)
1,5 DAN		utensilios de cocina de poliamida	<2.5	(Sanchis et al. 2015)

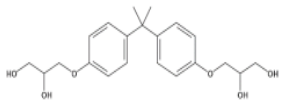
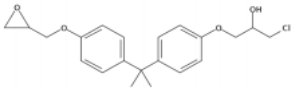
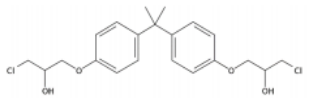
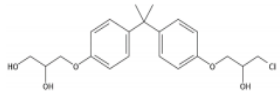
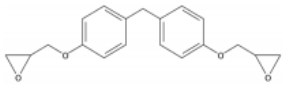
Teniendo en cuenta que muchas aminas aromáticas son compuestos tóxicos clasificados como presuntos carcinógenos en humanos (Sanchis et al. 2015, Brede et al. 2003), el Reglamento 10/2011 establece que cualquier material plástico no liberará aminas primarias en cantidad detectable en alimentos o simulantes. El LME se fijó a 0,01 mg de sustancias por el kilogramo de alimento o simulante y se aplica a la suma de aminas aromáticas primarias (ANL, 2-4 TDA, 2-6 TDA, 4-4MDA, 1-5DAN, m-PDA, 3-3DCB y 4-4’ DPE). En los últimos años el número de alertas alimentarias publicadas en el Sistema de Alerta Rápido para alimentos y piensos (RASFF) de la Unión Europea, ha aumentado de forma considerable en lo relativo a la detección de PAAs en utensilios de cocina de poliamida (RASFF, 2019).

Bisfenoles y compuestos relacionados

Los Bisfenoles y compuestos relacionados de mayor interés en seguridad alimentaria se recogen en la Tabla 6.

Tabla 6. Niveles, procedencia y estructura de Bisfenoles y compuestos relacionados

Fenoles	Estructura	Procedencia	Niveles (ng/g)	Referencia
BPA		Alimentos infantiles, biberones, papel reciclado, pescado, fruta y verdura	0.27(alimentos infantiles)-317 (fruta)	(Ballesteros-Gómez et al. 2009, Gallart-Ayala et al. 2011) .
BPS		Frutas y verduras, papel reciclado	11.5–175	(Pérez-Palacios et al. 2012)
BPF		Bebidas y papel reciclado	0.14–0.22 ng/mL	(Gallart-Ayala et al. 2011, Satoh et al. 2004)
BADGE		Papel reciclado, carne, pescado	0.1-11800	(Gallart-Ayala et al 2011, Míguez et al. 2012, Satoh et al. 2004)
BADGE·H_2O		Frutas y verduras	n.d-53 ng/g	(Gallart-Ayala et al 2011)

Fenoles	Estructura	Procedencia	Niveles (ng/g)	Referencia
BADGE· $2H_2O$		Pescado y frutas y verduras	0.6-106.4	(Gallart-Ayala et al 2011)
BADGE· HCl		Pescado y carne	0.3-477	(Gallart-Ayala et al 2011)
BADGE· 2HCl		Pescado y carne	0.8-155.2	(Gallart-Ayala et al 2011)
BADGE· H_2O·HCl		Pescado y carne	0.2-1085	(Gallart-Ayala et al 2011)
BFDGE		Papel reciclado, pescado	0- 4200	(Míguez et al. 2012)

El BPA se utiliza como monómero para la producción de policarbonato, resinas y papel térmico. Los datos de producción y consumo en Europa se detallan en la tabla 7 (INSTT 2011). Estudios recientes destacan el potencial del BPA para interrumpir la acción de la hormona tiroidea (disruptor endocrino), causar la proliferación de células cancerosas de la próstata humana y bloquear la síntesis de testosterona (Gao et al. 2013). El uso de BPA en los materiales en contacto con los alimentos está permitido en la Unión europea (UE) bajo el Reglamento 10/2011/EU para materiales plásticos y artículos destinados a entrar en contacto con productos alimentarios, con una LME de 0,6 mg/kg (EU 10/2011). La Comisión Europea aprobó la Directiva 2011/8/EU (CD 2011/8/EU) en enero de 2011, prohibiendo su uso para la fabricación de botellas de policarbonato en alimentación infantil (Gallart-Ayala et al. 2013, Núñez et al. 2012). El BPA también se ha encontrado en papel reciclado y cartón utilizado para el envasado de alimentos (pizza, bolsas de papel) y en las servilletas de cocina hechas de papel reciclado, probablemente debido a su uso en tintas de impresión (Gao et al. 2013). Por esta razón, es muy importante establecer los criterios para asegurar que el material utilizado para la fabricación de papel reciclado esté libre de contaminantes y sea utilizado para el envasado de alimentos.

Se necesitarían más datos para cuantificar el impacto de estas fuentes en la exposición a BPA en la población (Pérez-Palacios et al. 2012). La Agencia Europea de seguridad alimentaria realizó una evaluación del riesgo de BPA, estableciendo una ingesta diaria tolerable de 4 g/kg $_{\text{peso corporal}}$ (EFSA SO 2015)

Debido a la toxicidad del BPA y a las restricciones legales, se están utilizando otros compuestos análogos del BPA como sustitutos tales como, el bisfenol F (BPF) y el bisfenol S (BPS). La toxicidad del BPF y BPS está principalmente relacionada con los efectos estrogénicos y anti androgénicos (Satoh et al. 2004). A su vez, existen otros compuestos relacionados como el bisfenol A diglicidil éter (BADGE) y bisfenol F diglicidil éter (BFDGE). BADGE y BFDGE, tiene efectos citotóxicos, que los hacen tumorgénicos y mutagénicos (Míguez et al. 2012).

Los barnices y lacas de recubrimiento más utilizadas en latas de bebidas y alimentos están hechas con organosoles de vinilo (novolacs), que incluyen resinas epoxi obtenidas de BADGE o de BFDGE en su composición. Con respecto a los BADGEs, la UE ha fijado LME de 9 mg/kg para la suma de estos y sus derivados hidrolizados y 1 mg/kg para la suma de BADGE·HCl, BADGE·2HCl y BADGE·HCl·H2O (EU1895/2005).

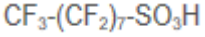
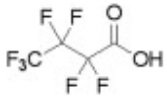
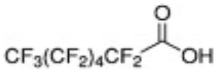
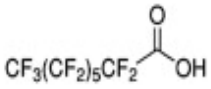
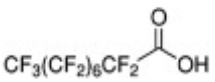
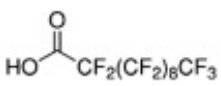
Tabla 7. Producción y consumo de bisfenol A (INSTT 2011)

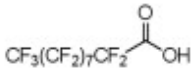
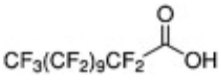
Uso	**Tm/año**	**Consumo en la UE (%)**
Producción de policarbonato	486.88	71.1
Producción de resinas epoxi	171.095	25
Resinas fenoplásticas	8.8	1.3
Resinas de poliéster insaturado	3	0.4
Revestimiento conservas	2.46	0.4
Producción de PVC	2.25	0.3
Producción de papel térmico	1.4	0.2
Fabricación de poliuretano	950	0.1
Fabricación de ruedas	110	<0.1
Producción de poliamida modificada	150	<0.1
Líquido de frenos	45	<0.1
Fabricación de bisfenol A alquiloxidado	2.02	0.3
Otros usos menores	5.990	0.9
Consumo UE	684.650	

Compuestos Perfluorados (PFCs)

Los compuestos Perfluorados (PFCs) son sustancias químicas persistentes y bioacumulables que provocan efectos adversos en la salud (Dallaire et al. 2009, Tao et al. 2008). En la Tabla 8 se detallan los PFCs que aparecen en los MCA.

Tabla 8. Niveles, procedencia y estructura de PFCs

PFCs	Estructura	Procedencia	Niveles [(1)](ng/g)	Referencia
PFOS		Alimentos infantiles, biberones, pescado y carne en conserva, fruta y verdura, papel reciclado	0.003 (carne enlatada)-12.8 (conservas de pescado)	(Moreta et al 2014, Ballesteros-Gómez et al. 2010, Jogsten et al. 2009, Pico et al. 2011)
PFBA		Espinacas envasadas, papel reciclado	n.d-280	(Ballesteros-Gómez et al. 2010, Hrádková et al. 2010)
PFPeA		Bebidas y papel reciclado	n.d-43	(Zafeiraki et al. 2014)
PFHxA		Papel reciclado, espinacas envasadas	2.2-497	(Moreta et al 2014, Zafeiraki et al. 2014, Jogsten et al. 2009)
PFHpA		Papel reciclado, espinacas envasadas, carne	1.18-7.6	(Zafeiraki et al. 2014, Jogsten et al. 2009)
PFOA		Pescado y carne enlatada, papel	n.d-1.7	(Moreta et al 2014, Raymer et al. 2012)
PFNA		Leche y derivados	n.d-0.5	(Wang et al. 2010)
PFUnA		Leche y derivados	0.015- 0.040	(Wang et al. 2010)

PFCs	Estructura	Procedencia	Niveles (1)(ng/g)	Referencia
PFDA		Pescado y carne, alimentos infantiles	n.d-1.3	(Moreta et al 2014, Ballesteros-Gómez et al. 2010, Jogsten et al. 2009, Pico et al. 2011)
PFDoA		Papel reciclado, espinacas envasadas	0.045–0.075	(Zafeiraki et al. 2014, Jogsten et al. 2009)

(1): Niveles descritos en la bibliografía en alimentos o en ensayos de migración.

Estas sustancias comprenden un amplio grupo de compuestos que han sido utilizados en utensilios de cocina y materiales de contacto con alimentos, debido a sus propiedades químicas tales como su estabilidad térmica, además de su naturaleza hidrofóbica (Buck et al. 2011). Entre los PFCs se encuentran los poliperfluoroalquil ácidos sulfonados (PFSAs) y los poliperfluoroalquil acidos carboxilados (PFCAs) de los cuales el perfluorooctano sulfonato (PFOS) y el ácido Perfluorooctanoico (PFOA) son los más conocidos (Kannan et al. 2004). El PFOS ha sido incluido en el Convenio de Estocolmo sobre contaminantes orgánicos persistentes (COP), prohibiéndose su producción y uso (UNEP 2009).

La exposición directa a estos compuestos puede darse debido a su fabricación y uso en productos comerciales (Sinclair et al. 2007) y, por otro lado, la exposición indirecta puede darse debido a la liberación de los propios precursores en el ambiente durante la producción (D'eon et al. 2006, Martin et al. 2006, Prevedouros et al. 2006, Beser et al. 2019).

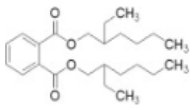
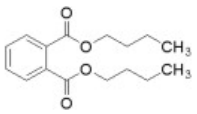
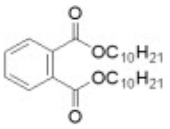
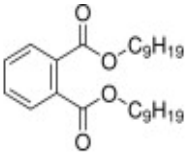
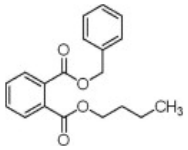
Ejemplos de los precursores podrían ser: alcoholes fluorotelomeros (FTOHs), perfluorooctanosulfonamidas (FOSAs) y perfluorooctanoetanol sulfonamidas (FOSEs). Los ésteres del fosfato de polifluoro alquilo (PAPs), usados principalmente en materiales en contacto con alimentos y que son precursores de FTOHs y posteriormente también de PFOA (Lee et al. 2010). Recientemente estos compuestos se han convertido en un foco de atención debido a su amplio uso en los materiales de empaquetado hechos del papel y de cartulina, incluyendo el papel de embalaje, en la leche y los cartones de zumos, los envases de la comida rápida y las bolsas de las palomitas de la microondas. La EFSA completó la evaluación del riesgo de PFOS y PFOA en la cadena alimentaria y estableció una tolerancia de ingestión diaria admisible (IDA) de 150 ng/kg de peso corporal/día y

1500 ng/kg de peso corporal/día, respectivamente para estos compuestos (Gallart-Ayala et al. 2013).

Ftalatos (PAEs)

Los ftalatos (PAEs) de mayor interés en seguridad alimentaria se encuentran en la tabla 9.

Tabla 9. Niveles, procedencia y estructura de PAEs.

PAEs	Estructura	Procedencia	Niveles(1) (µg/kg)	Referencia
DEHP		Leche y derivados, envases plástico y cartón.	1-936	(Jia et al. 2014, Fasano et al. 2012) .
DBP		Alimentos infantiles envases plásticos	5.6–9.9 ng/mL	(Xu et al. 2014, Mortensen et al. 2005)
DIDP		Leche y derivados, bolsas	n.d- 5	(Jia et al. 2014, Fromme et al. 2007)
DINP		Leche y derivados, bolsas	n.d- 5	(Jia et al. 2014, Xu et al. 2014, Fierens et al. 2012, Sanchis et al. 2018, Sagratini et al. 2008)
BBP		Gelatinas de fruta	2900–14700	(Magi et al. 2010)

(1): Niveles descritos en la bibliografía en alimentos o en ensayos de migración.

Los ftalatos (PAEs) o los ésteres del ácido ftálico se han utilizado comúnmente como plastificantes para aumentar flexibilidad, la transparencia, la durabilidad, y la longevidad de los materiales plásticos desde los años 30 (Farahani et al. 2008). Millones de toneladas de PAEs se producen anualmente en todo el mundo, de los cuales el dietilhexilftalato (DEHP) es uno de los plastificantes más populares y cuenta con aproximadamente el 50% de la producción global, seguida de dibutiloftalato (DBP), di-isodecil ftalato (DIDP) y di-iso-nonil ftalato (DINP) (Gallart-Ayala et al. 2013, Espachs-Barroso et al. 2005). En general, el contenido de PAEs en materiales plásticos, tales como el policloruro de vinilo (PVC), polietilenttereftalato (PET), polivinilacetato (PVA) y polietileno (PE), varía de 10% a 60% en peso (IARC 2016).

Los ftalatos y sus metabolitos pueden causar efectos perjudiciales a la salud humana. Por ejemplo, los investigadores demostraron que el DnBP, el BBP, el DEHP y el DiNP pueden afectar adversamente al sistema reproductivo masculino. Duty et al. (2003) encontró una asociación entre el ADN dañado en espermatozoides y la exposición al DEP. Además, Latini et al. (2004) revelaron que el DEHP puede interrumpir el sistema endocrino en seres humanos.

Los ftalatos pueden migrar fácilmente de los materiales de envasado de alimentos en ciertas condiciones, particularmente cuando están en contacto con alimentos grasos y aceitosos (Fan et al. 2014, Dodson et al. 2012).

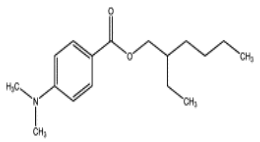
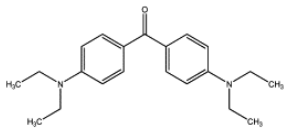
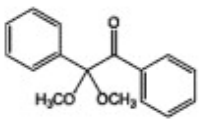
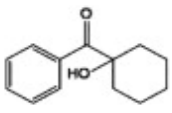
La exposición humana a ftalatos se produce principalmente a través de la ingesta de alimentos (Frommer et al. 2007, Fasano et al. 2012). Por ejemplo, en 2011, seis ftalatos fueron detectados en muestras de leche envasada con plásticos (Yan et al. 2011). Además de la leche líquida, Ostrovský et al. (2011) encontró que la leche en polvo también se contaminó a DEHP con alta concentración. Sin embargo, los ftalatos en los alimentos no tuvieron especial atención hasta 2011 cuando en Taiwán se encontró que DEHP estaba siendo utilizado ilegalmente en bebidas, resultando de gran preocupación para la salud pública (Espachs-Barroso et al. 2005, NSOPC 2011). En vista de los peligros potenciales para la salud humana y animal, algunos ftalatos (por ejemplo, DMP, BBP, DBP, DEP, DNOP y DEHP) han sido catalogados como contaminantes prioritarios y su uso en alimentos y productos plásticos ha sido restringido en la Unión Europea (EU), Estados Unidos y China (NSOPC 2011). Utilizando alimentos simulantes, la Unión Europea estableció LME para varios ftalatos en materiales en contacto con alimentos (EFSA 2005a, EFSA 2005b, EFSA 2005c, EFSA 2005d, EFSA 2005e, EU 2007/19/EC 2007).

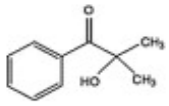
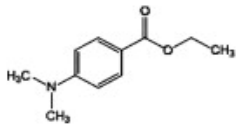
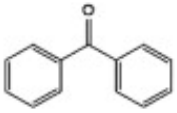
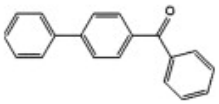
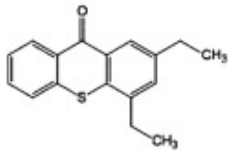
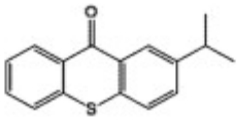
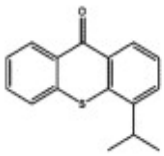
Por ejemplo, el LME (mg/kg simulante) para BBP, DEHP y DBP son 30, 1,5 y 0,3 mg/kg, respectivamente (Kenneth 2007). Con respecto a los sin LME, se aplica un límite de 60 mg/kg en productos alimentarios (EFSA 2004).

Fotoiniciadores

Los fotoiniciadores más relevantes en los materiales destinados al envasado o a la protección de los alimentos se encuentran en la Tabla 10.

Tabla 10. Niveles, procedencia y estructura de los fotoiniciadores

Fotoiniciadores	Estructura	Procedencia	Niveles [1]	Referencia
EHDAB		Leche y derivados, tetrabrik, bolsas para zumo	0.13–120 ng/g	(Sagratini et al. 2008, Sanchis et al. 2018) .
DEAB		Zumos de fruta, tetrabrik, bolsas para zumo	n.d[a]-0.7 ng/mL	(Sanchis et al. 2018, Suciu et al. 2013)
DMPA		Alimentación infantil, Zumos de fruta, tetrabrik	n.d[a]-0.2 ng/g	(Gallart-Ayala, et al. 2011, Sagratini et al. 2008, Shen et al. 2009)
HCPK		Vinos, Zumos de fruta, tetrabrik	n.d[a]- 1.2 ng/mL	(Sagratini, ¡ et al. 2008, Sanchis, ¡et al. 2018, Gallart-Ayala et al. 2011)

Fotoiniciadores	Estructura	Procedencia	Niveles (1)	Referencia
HMPP		Tetrabriks, leche, vino, carne, Zumos de fruta, tetrabrik	n.d[a]	(Sagratini et al. 2008, Sanchis, et al. 2018, Gallart-Ayala et al. 2011)
EDMAB		Zumos de fruta, tetrabrik,	0.5-2.5 ng/mL	(Sagratini et al. 2008, Sanchis et al. 2018, Gallart-Ayala et al. 2011)
BFN		Tetrabriks, leche, vino, carne, Zumos de fruta, tetrabrik.	2 - 350 ng/cm	(Sagratini et al. 2008, Sanchis et al. 2018, Gallart-Ayala et al. 2011, Shen et al. 2009)
PBZ		Tetrabriks, leche, vino, carne, Zumos de fruta, tetrabrik	n.d[a]	(Sagratini et al. 2008, Sanchis et al. 2018, Gallart-Ayala et al. 2011)
DETX		Alimentos infantiles, zumos de fruta, tetrabrik.	0.1 ng/g, 0.07 ng/mL	(Sanchis et al. 2018, Sagratini et al. 2008, Suciu et al. 2013)
2-ITX		Leche y derivados lácteos, Zumos de fruta, tetrabrik	0.81–439 ng/g	(Sanchis et al. 2018, Aznar et al. 2015, Shen et al. 2009)
4-ITX				

a: no detectado, (1): Niveles descritos en la bibliografía en alimentos o en ensayos de migración.

Los fotoiniciadores conforman las tintas que se utilizan comúnmente en los materiales para envasado de alimentos. Cuando se utilizan materiales multicapa, formados por varias capas de PE, PRP o incluso papel reciclado, la migración puede realizarse, no sólo desde la superficie de contacto directo con los alimentos sino también desde capas más internas debido a procesos de difusión y partición (Aznar et al. 2015). En el caso de las tintas aplicadas en el lado externo del envase, puede haber transferencia de la tinta del lado externo al lado interno durante la producción, y durante el almacenado aumenta la posibilidad de que el componente de la tinta migre al alimento.

El uso de las tintas de impresión para envasado de alimentos está regulado por la Asociación Europea de tintas de impresión (EuPIA) (EUPIA 2013). Diversos grupos de materias primas se pueden utilizar en la fabricación de tintas para envasado de alimentos tales como, colorantes, pigmentos, tintes, aditivos del pigmento, resinas poliméricas, solventes o fotoiniciadores (EUPIA 2013). Aunque las capas de aluminio intermedias se utilicen comúnmente para prevenir la migración de los componentes de la tinta en productos alimenticios, la transferencia no intencionada de los componentes de la tinta de impresión de la superficie impresa externa sobre la superficie del alimento en contacto puede ocurrir cuando está impresa. Aunque los fotoiniciadores de tinta son ampliamente utilizados, no hay controles específicos en la Unión Europea para la migración de las tintas y recubrimientos, aunque sí que se ha establecido el límite de migración específica LME para benzofenona de 0,6 mg/kg en una legislación específica para los plásticos en contacto con los alimentos (Kenneth et al 2007). Con respecto a los que no tienen un límite, se aplica un LME de 60 mg/kg.

Aditivos

Aditivos tales como los antioxidantes, los estabilizadores y los plastificantes como la cloro parafina, tienen una influencia importante en el proceso y la vida útil de los plásticos, y son responsables de muchas características de estos materiales. Estos aditivos están presentes en pequeñas cantidades en plásticos (generalmente con porcentajes entre 0,1- 1%). Están dispersos en la matriz polimérica, con el principal objetivo de evitar efectos tales como el deterioro termo-oxidativo, que inicia la escisión y reticulación de las cadenas macromoleculares y conducen consecuentemente al deterioro del polímero (EU 2011). El polímero tiene una estructura inerte con un alto peso molecular que representa un riesgo potencial bajo para la salud puesto que el organismo no puede absorber las moléculas con un peso molecular mayor que 1000 Da (Bignardi et al. 2014). Por el contrario, a medida que los aditivos plásticos y los colorantes orgánicos tienen un peso molecular más bajo, pueden migrar de los plásticos a los alimentos, lo que sí representa un riesgo para la salud humana (Bignardi et al. 2014).

Sustancias añadidas de forma no intencionada (NIAS)

Los alimentos envasados pueden contener sustancias añadidas no intencionadamente (NIAS) como resultado de las interacciones entre diferentes ingredientes del envase, por los procesos de degradación y liberación de monómeros que conforman el polímero del

envase, y principalmente a las impurezas presentes en las materias primas utilizadas en la producción del envase (Aznar et al. 2012). La mayoría de los compuestos NIAS desconocidos son a menudo detectados al usar técnicas analíticas de alta sensibilidad y con una base de datos para realizar análisis retrospectivo. En la mayoría de los casos, debido a los bajos niveles encontrados, estas sustancias no son de especial preocupación para la salud. Sin embargo, en la actualidad no existe ninguna guía sobre el análisis de sustancias desconocidas en este campo. La regulación sobre los materiales del contacto del alimento (Reglamento10/2011/EU) (EU 10/2011) reconoce que, durante la fabricación y uso de materiales y artículos plásticos, pueden formarse NIAS. Su contribución puede ser relevante para la evaluación del riesgo. Sin embargo, la identificación de NIAS es muy difícil debido a la falta de información sobre la composición real de los diversos ingredientes y materiales utilizados para los polímeros y la fabricación de envases finales.

Con el fin de evitar la presencia de NIAS en los alimentos envasados, especialmente cuando hay una evaluación del riesgo, la trazabilidad de los materiales de envasado de alimentos es obligatoria y esto incluye una descripción de NIAS (Nerin et al. 2013). Los procesos de degradación y las impurezas, como se ha dicho, son fuentes importantes de NIAS. Los procesos de degradación pueden tener lugar en el propio polímero y también en los aditivos utilizados para mejorar sus características fisicoquímicas. Algunos aditivos utilizados como antioxidantes o estabilizadores añadidos al polímero para mejorar sus propiedades también pueden degradarse. Como resultado, los nuevos migrantes potenciales estarán presentes en el material cuando se produzca su envejecimiento. Algunos de los productos más comunes de la degradación que se han estudiado debido a su toxicidad son: alquilfenol, nonilfenol (NP) y Octilfenol (OP), que son disruptores endocrinos (Bach et al. 2012). NP y OP pueden ser generados por la oxidación del tris (nonilfenil) fosfato (TNPP), utilizado como antioxidante en materiales poliméricos como el PVC. Pueden también ser generados por la degradación de nonilfenil polietoxilado (APEO), usados en la fabricación de botellas para animales domésticos (tereftalato de polietileno) y en otros materiales tales como pegamentos (Nerin et al. 2013). También se han encontrado impurezas de los aditivos en adhesivos utilizados en envases en la migración. Los compuestos 1-hexanol-2-etil, 2-ethilhexilacetato y 2, 4, 7, 9-tetrametil-5-decilglucósido-4, 7-diol (TDMM) fueron encontrados en la migración de materiales multicapa, exactamente en los pegamentos. Los primeros dos compuestos son

impurezas del 2-etilhexilacrilato, un monómero usado en la producción de pegamentos de acrílico, y el último era una impureza o un monómero residual del tensioactivo de etoxilato TDMM, usado como adhesivo (Canellas et al. 2010).

1.2. Metodologías analíticas

El análisis de contaminantes procedentes de los MCA se puede realizar en los alimentos o en el propio material. La determinación del tipo de análisis viene dada por las condiciones en las que se encuentra el material, de acuerdo con el Reglamento de la UE 1935/2004. Si ya ha entrado en contacto con el alimento el análisis se realizará en el alimento; si por el contrario no se ha puesto en contacto, pero se conoce para qué alimento está destinado, el análisis se realizará a través de simulantes. Únicamente se realiza directamente en el material en las industrias de fabricación si no es conocido su destino.

En la presente tesis se ha realizado una revisión bibliográfica de las metodologías analíticas para la determinación de contaminantes en MCA. Las tablas 11 y 12 muestran los procedimientos analíticos basados en la cromatografía de gases (tabla 11) y la cromatografía líquida (tabla 12) para los principales grupos de sustancias en las tres matrices estudiadas, alimentos, simulantes y envases. Un esquema general de las metodologías analíticas para los contaminantes en alimentos y envases se muestra en las figuras 3 y 4.

Tabla 11. Revisión de las metodologías analíticas mediante GC para la determinación de contaminantes de materiales en contacto con los alimentos.

Compuestos	**Alimento**	**Material en contacto**	**Extracción**	**GC condiciones (Columna/ fuente y analizador)**	**LD**	**LC**	**Recuperación**	**Referencias**
Ftalatos, adipatos, sebacatos: DEA, DiBA, DBA, DEHA, DMP, DEP, DiBP, DBP, BBP,DiHP, DEHP, DOP, DiNP,DiDP, DEHS	aceite de oliva		liquido liquido con acetonitrilo	Supelco SPB-5MS (5%POLYDIPHENYLSILOXANE,95% POLYDIMETHYLSILOXANE) (0.25µmx30x0.25mm), EI modo (70eV), HRGC-MS Shimadzu QP5050	LD (mg/kg): 0.04-1.200	LC (mg/kg): 0.040- 4	93-101%	(Dugo et al. 2011)
Na, 2MNa,1MNa, 26DMNa,16DMNa,14 DMNa,12DMNa, Acy ,DMP,Ace, DEP, Fln ,27DiPNa, 26DiPNa, BFN,1MFln,EDB, 4MBFN, DiBP, Phe,Ant, DBP, 2MAnt ,9MAnt, Ant,DNpp, Flt, DEHA ,Pyr, EHDB, DEHP,DiNP, DHpP, 4ITX, 2ITX,DiDP, BaA,DnOP,Chr,BbF,B kF,BaP, IcdP, BghiP.		papel	con QuEChERS	Rxi-PAH columna (0.10µmx30x0.25mm), EI modo (-70eV), GC-MS/MS, Trace 1310 GC.	-	LQ (mg/kg): DnOP 0.011, -BPF 0.070.	52-135 %	(Vavrouš et al. 2016)

Compuestos	Alimento	Material en contacto	Extracción	GC condiciones (Columna/ fuente y analizador)	LD	LC	Recuperación	Referencias
Ftalatos: dimetil ftalato (DMP), dietil ftalato (DEP), diisobutil ftalato (DiBP), di-n-butil ftalato (DnBP), benzilbutil ftalato (BBP), di(2-etilhexil) ftalato (DEHP), diciclohexil ftalato (DCHP) y di-n-octil ftalato (DnOP)	Vegetales y frutas, leche y otros productos, cereales, carne, pescado, grasas y aceites, bebidas, snacs y alimentos infantiles		LIQUIDO -LIQUIDO, purificación por cromatografia de permeación en gel (GCP)	DB-XLB columna (0.25µmx60x0.25mm), EI/CI MSD modo, GC-EI-MS, cromatografia de gases- baja resolución-espectrometria de masas.		LC (µg/kg): DMP:(0.01- 145 DEHP)	(DMP:(93%), DEP (98%), DiBP (101%), DnBP (99%), BBP (95%), DEHP (100%), DCHP (89%), DnOP (94%)	(Fierens et al. 2012)
ftalatos: dimetil ftalato (DMP), dietil ftalato (DEP), diisobutil ftalato (DiBP), di-n-butil ftalato (DnBP), benzilbutil ftalato (BBP), di(2-atilhexil) ftalato (DEHP), diciclohexil ftalato (DCHP) y di-n-octil ftalato (DnOP)		Carton, Tetra Brick, Plasticos	Extraction 60 min con 40 ml n-hexano in an ultrasonic en baño con ultrasonidos	DB-XLB columna (0.25µmx60x0.25mm), EI/CI MSD modo, GC-EI-MS, cromatografia de gases- baja resolución-espectrometria de masas.		LC (µg/kg) (DMP:(0.1)- DnBP (1.5))	(DMP:(90%), DEP (85%), DiBP (82%), DnBP (82%), BBP (85%), DEHP (99%), DCHP (91%), DnOP (90%)	(Fierens et al. 2012)
Ftalatos y adipatos: DEP, BBP, DEHP, DBP, and DOP, DEHA, DiBP, DCHP	muestras de carne (pollo asado)		microextracción en fase solida con fibra enfriada por corriente de nitrogeno CF-SPME)	HP-5MS Agilent columna (0.25µmx30x0.25mm), EI modo (70eV), GC-MS, (7890C Agilent)	LD (µg/kg): DBP (0.01)- BBP (0.18)	LC (µg/kg): DiBP (0.07) - BBP (0.26)		(Moreira et al. 2015)

Compuestos	Alimento	Material en contacto	Extracción	GC condiciones (Columna/ fuente y analizador)	LD	LC	Recuperación	Referencias
Ftalatos y esteres: DEP, DEHP,DBP, y DnOP, DMP,BBP	78 muestras representativas de consumo		extracción en fase solida con purificación usando purga de gas con micro jeringa extracción (GP-MSE)	DB5 fused-silica columna capilar(0.25µmx30x0.25mm), EI modo (70eV), GC-MS con ionización por electro impacto (Shimadzu GC 2010 System)	LD (ng/g) para sólido y (ng/ml) para liquido: (0.14- 0.18 ng/g-0.0021- 0.0038 ng/ml)		DEP:95%, DMP 92%, DBP 100%, BBP 90%, DEHP 100%, DNOP 93%	(Moreira et al. 2015)
DMP, DEP, DBP, BBP, DIOP, DNOP	leche embotellada		ultrasonidos asistidos por micro extracción liquido liquido dispersa	FID KB-1 (0.25µmx30x0.25mm), SPLIT RATIO, GC-MS	0.64-079ng/g		93.0-105.7%	(Yan et al. 2011)
DMP, DEP, DIBP, DBP, DAP, DHP, BBP, BBEP, DEHP, DOP	aceite de oliva		extracción en fase solida con espacio de cabeza	IT/MS ZB-5MS (0.25µmx30x0.25mm), GC-MS		0.02--0.05 mg/kg		(Rios et al. 2010)
DMP, DBP, DEP, DEHP, DNDP	grasas		extracción liquido-liquido	FID and MS DB-5 MS (0.25µmx30x0.25mm), SPLITLESS modo, GC-MS	0.4µg/g	1.2µg/g		(Yan et al. 2011)
DMP, DBP, DEP, BBP, DIBP, DEHP	vino		micro extracción liquido liquido dispersiva asistida por ultrasonidos y vortex	FID y IT/MS SE-54 (0.25µmx30x0.25mm), SPLITLESS modo, GC-MS	0.0022 µg/L	0.075 µg/L	85-100.5%	(Cinelli et al. 2013)
DMP, DEP, DBP, DAP, DNOP	leche de soja		extracción en fase con polímeros impresos (MISPE)	MS DB-5MS (0.25µmx30x0.25mm), SPLITLESS modo, GC-MS	0.013-0.022µg/mL		75.8-107.8 %	(He et al. 2010)

Compuestos	Alimento	Material en contacto	Extracción	GC condiciones (Columna/ fuente y analizador)	LD	LC	Recuperación	Referencias
DMP, DEP, DBP, BBP, DIBP, DEHP	comida y bebida con alcohol		extracción en fase solida (SPE) con Amberlite XAD-2 adsorbente.	FID y IT/MS SE-54 (0.24µmx30x0.25mm), SPLITLESS modo, GC-MS	1.21-2.51pg/µl	2.42-5.03 pg/µl	94-103 %	(Cinelli et al. 2014)
DBP, DEHP	sopa de pollo		micro extracción en fase solida con campo magnético	FID DB-5(0.25µmx30x0.25mm), GC-MS	26.3-36.4 µg/ml		70-118 %	(Makkliang et al. 2015)
DMP, DEP, DBP, BBP, DEHP, DOP	Vegetales y frutas, leche y otros productos, cereales, carne, pescado, grasas y aceites, bebidas, snacks y alimentos infantiles		micro extracción en fase solida	MS DB-17 MS (0.25µmx30x0.25mm), SPLITLESS modo, GC-MS	15.8-106pg/g		83-118.5 %	(Cacho et al. 2012)
DMP, DEP, DBP, DIBP, DMEP, DPEP, DNHP, DPHP, DNOP	grasas		extracción en fase solida dispersiva	MS DB-5 MS (0.25µmx30x0.25mm), SPLITLESS modo, GC-MS	0.4-0.8µg/ml		70.9-115.5%	(Zhu et al. 2013)
DMP, DEP, DBP, DIBP, DMEP, BBP, DHXP, DPEP, DCHP, DNP, DNOP	alimentos diversos		liquido-liquido, permeación en gel	MS DB-5 MS (0.25µmx30x0.25mm), SPLITLESS modo, GC-MS	1.5 mg/kg (grasa) 0.05 mg/kg (alimentos no grasos)			(Standard of the People's Republic of China, Determination of phthalate esters in foods. GB/T 21911-2008)
Etill carbamato	bebida alcohólica soja		extracción en fase solida	DB Innowax capillary (1.25µmx30x0.25mm), SPLITLESS modo, GC-MS		5µg/kg	96-107%	(Huang et al. 2013)

Compuestos	Alimento	Material en contacto	Extracción	GC condiciones (Columna/ fuente y analizador)	LD	LC	Recuperación	Referencias
Irgafos 168, Irganox 1076, Tinuvin 326, Chimassorb 81		films de PRP	24 hr a 60ºC, inmersión total con hexano	La precolumna no recubierta está conectada a una pieza en T metálica que une la salida de vapor del solvente y la columna de separación de 12 mx 0,25 mm recubierta con una película de 0,13 µm de PS-255, un dimetil polisiloxano reticulado. (Fluka), detector con ionización por llama (FID), HPLC-GC	0.1-1 mg/kg			(Castillo et al. 2013)
BPA, DEHP, NMP, NDP		cartón	BPA-extracción con etanol, DEHP, NMP y NDP in acetona-hexano 4:1, extracción Soxhlet	SLB-5ms tipo (5%polysilarylene-95% polydimethylsiloxane) (0.25µmx30x0.25mm), GC-MS	LD (mg/l): BPA 0.015- NMP 0.033,	LC (mg/l): DEHP 0.050 - NMP 0.1	101-108%	(Suciu et al. 2013)
BPA, DEHP, NMP, NDP	azúcar y sal		extracción con simulante Tenax	SLB-5ms tipo (5%polysilarylene-95% polydimethylsiloxane) (0.25µmx30x0.25mm), GC-MS	LD (mg/l): BPA 0.015- , NDP 0.033	LC (mg/l): DEHP 0.050- NDP 0.1	70-120%	(Suciu et al. 2013)

Compuestos	Alimento	Material en contacto	Extracción	GC condiciones (Columna/ fuente y analizador)	LD	LC	Recuperación	Referencias
Ethyl acetate, Methyl methacrylate, Toluene, Hexanal, Paraldehyde, P-xylene, Butyl acrylate, Styrene, P-cymene, 2-octanone, 1-hexanol, 2-ethylhexylacetate, Nonanal, Cyclohexanol, Acetic acid , 2-ethyl-1-hexanol, Camphor, Propanoic acid, Benzaldehyde, 1-octanol, butyric acid, Methylbenzoate, naphtalene, allylbenzoate.		materiales multicapa para comida seca	migración con Tenax en el horno a 40ºC durante 10 días	BP-20 (0.25µmx30x0.25mm), SPLITLESS modo, GC-O-MS	LD (µg/g) valores entre 0.01(tolueno) y 25.7 (acido acético).			(Vera et al. 2014)
compuestos volatiles: hexanal, octanal, 2-heptenal, 1-hydroxy-2-propanone, nonanal, 2-octenal, furfural, decanal, 2-nonenal, 2-furanmethanol, 2-decenal, 2,4 decanienal isomers, hexanoic acid	leche en polvo		Migración en horno a 40ºC durante 10 días.	DB WAX (0.20µmx50x0.40µm) , GC-MS				(Francesca et al. 2015).
MELAMINA	leche en polvo		extracción liquido-liquido	HB-5MS (0.25µmx30x0.25mm), EI modo (70eV), GC-MS		0.025mg/kg	95-101%	(Lutter et al. 2011).

Compuestos	Alimento	Material en contacto	Extracción	GC condiciones (Columna/ fuente y analizador)	LD	LC	Recuperación	Referencias
FTMAC, FTACs, FTOHs, FOSAs, y FOSEs			dos extracciones, primero acético 3%, después metanol, después purificación SPE (extracción en fase solida)	DB-WAX columna (0.25µmx30x0.25mm), usando ionización química positiva (PCI), GC-PCIMS	LD (pg) valores entre 3.9-30pg			(Francesca et al. 2015)
133 compuestos volátiles (esteres, ácidos, alcoholes saturados, alcoholes alifáticos insaturados , cetonas ,aldehídos, furanos, éteres, lactonas, compuestos que contienen sulfuros)	leche de camello		tres métodos de pretratamiento de micro extracción en fase sólida, solvente, destilación simultánea a la extracción	DB-WAX columna (0.25µmx30x0.25mm), SPLITLESS modo, GC-MS				(Ning et al. 2011)

Tabla 12. Revisión de las metodologías analíticas mediante LC para la determinación de contaminantes de materiales en contacto con los alimentos.

Compuestos	**Alimento**	**Material en contacto**	**Extracción**	**LC condiciones (Columna/Fuente de ionización y técnica)**	**LD**	**LC**	**Recuperación**	**Referencias**
Aminas: ANL,2,4 TDA, 2,6 TDA, m-PDA, 1,5 DAN, 3,3' DMB, 4,4'DPE,4,4' MDA		utensilios de cocina de poliamida	Ácido acético 3%,2hrs a100ºC	C18-Phenyl hexil columna (2.1µm x 100x2.1mm), Metanol 2%/Agua 98%, ESI (+), LC-HRMS (orbitrap)		2.5µg/kg	70-120%	(Sanchis et al. 2015)
Aminas: ANL, 2,4 TDA, BNZ, o-ANS,4,4' DPE, o-TOL, 4,4' MDA, o- DANS, o- TOLI, p-ANL, p-CRS, 4,4' MBM,4,4' TDA, 2-NAPH, 4-Cl-TOL, 5-N-O-TOL, 2,4,5 MTA, 4- ABF,4,4' M(2Cl),3,3' DCB, p-ABZ, o-AaT		láminas de plástico	0.6 dm de láminas de plástico fueron cortadas e inmersas en 100ml de ácido acético al 3% la solución se incubó a 70ºC durante 2 h.	C18 (2.6µmx100x2.1mm) agua 4.7mM ácido perfluorpropanoico (PFPA)/4.7mM PFPA en metanol, ESI (+), HPLC-HRMS (orbitrap)	0.06-5.27µg/kg	0.09-5.45µg/kg		(Mattarozzi et al. 2013b)
BPA, BADGEs y compuestos relacionados: BPA, BPF, BADGE, BFDGE		papel reciclado	extracción solida liquido enfocada con ultrasonidos (FUSLE)	C18 (1.7µmx50x2.1mm) ACN: agua/0.5mM acetato sódico 8.5mM acido acético, ESI (+), UPLC-Q-TOF-MS	16-47 ng/ml		72-97%	(Pérez-Palacios et al. 2012)
BPA, BPB, BPF, BPE, BFDGE, BADGE, BADGE-H2O, BADGE-2H2O, BADGE-H2O-HCl, BADGE-HCl, BADGE-2HCl, BFDGE-2HCl	alimentos enlatados		Micro extracción solido-liquido	C18 (5µmx250x4.5mm) ACN/agua en condiciones de isocrático, Cromatografía liquida por fluorescencia		0.9 - 3.5 µg/kg	80–110%	(Alabi et al. 2014)

Compuestos	Alimento	Material en contacto	Extracción	LC condiciones (Columna/Fuente de ionización y técnica)	LD	LC	Recuperación	Referencias
BPA, BADGE		policarbonato	cloroformo/metanol	C18 Acclaim PepMapRSLC (2µmx 150x0.3mm)1 mM formiato amónico en 10:90 metanol: agua (v/v)/1 mM formiato amónico en metanol, ESI(+)(-),UHPLC			95-98%	(Bignardi et al. 2014)
Compuestos perfluorados Acidos: Perfluorooctanosulfonico sal tetraetilamonio PFOS, perfluorobutanoico PFBA, ácido perfluoropentanoico PFPeA, perfluorohexanoico PFHxA, perfluroheptanoico, PFHpA, perfluorooctanoico PFOA, perfluorononanoico PFNA, perfluorodecanoico PFDA, perfluoroundecanoico PFUnA, perfluorododecanoico PFDoA		bolsas de palomitas para horno	extracción solida liquido asistida por ultrasonidos (FUSLE)	C18 (1.7µmx50x2.1mm) 0.8% fórmico-Acetonitrilo/ 0.8% fórmico, ESI (-), UHPLC-QTOFMs/Ms análisis	0.2-0.5 ng/g	0.4-1.6 ng/g	80-106 %	(Moreta et al 2014)
Compuestos perfluorados: PFOS, PFBA, PFPeA, PFHxA, PFHpA,PFOA, PFNA, PFDA, PFUnA, PFDoA	palomitas de maíz y maíz		extracción solida liquido asistida por ultrasonidos (FUSLE)	C18 (1.7µmx50x2.1 mm) 0.8% fórmico-Acetonitrilo/ 0.8% formico), ESI (-), UHPLC-QTOFMs/Ms	0.2-0.7 ng/g	0.2-0.6 ng/g	65-105 %	(Moreta et al 2014)

Compuestos	Alimento	Material en contacto	Extracción	LC condiciones (Columna/Fuente de ionización y técnica)	LD	LC	Recuperación	Referencias
Compuestos perfluorados: PFBA, PFOA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDOA, PFBS, PFHxS, PFOS, PFTrDA, PFTeDA, PFHxDA, PFODA, PFDS		hojas de aluminio, tazas de bebida y envases de papel	extracción liquida presurizada (PLE)con metanol y purificación con Florisil-Basico y columna de alúmina	Hypersil GOLD C8 (3µm,150 mm, 2.1 mm) 5 mM acetato amónico – MeOH, LC-MS/MS	desde 0.20 a0.94 ng/g		60-90%	(Zafeiraki et al. 2014)
PFCs, PFCA, PFAAS, PFAPA, PAP, FOSA, y bisfenoles		envases de papel, papel para productos de horno, folios de papel para almacenar comida, cajas de cartón, filtros de papel para el café.	extracción con ultrasonidos con mezcla de acetonitrilo y agua seguida por QuEChERS	EC-C18 columna (2.7µm,150 mm,3 mm), HPLC- MS/MS		0.0027-0.13 mg/kg	70-120%	(Vavrouš et al. 2016)
Compuestos perfluorados:PFOS, PFBA,PFPeA, PFHxA , PFHpA,PFOA, PFNA, PFDA, PFUnA, PFDoA	huevos y pollo		extracción solido-liquido con metanol	C18 columna (5µm, 50 mm,2.1mm)/ formiato amónico en agua/ metanol, LC-MS/MS	0.15 ng/g	0.5 ng/g	90-120%	(Zafeiraki et al. 2016)

Compuestos	Alimento	Material en contacto	Extracción	LC condiciones (Columna/Fuente de ionización y técnica)	LD	LC	Recuperación	Referencias
Ftalatos:DMP, DMEP, DEEP, DEP, DAP, DIPrP, DPrP, DPhP, DIBP, BBP, DIPP, DPP, DCHP, DMPP, DHXP, DHP, DEHP, DNOP, DINP, DNP, DIPP.	alimentos incluyendo: derivados lácteos, licor destilado, vino, bebidas, cereales, carne, aceite, galletas, y comida enlatada		Muestras liquidas extraídas con acetonitrilo. Muestras solidas extraídas por QuEChERS basado en métodos SPE	Poroshell 120 EC-C18 columna (100 × 4.6 mm, 2.7µm), LC-MS/MS	0.8-15 µg/kg	10-100 µg/kg	75.5-115.2 %	(Xu et al. 2014)
Ftalatos: DMP, DEP, DiBP, DnBP, BBP, DEHP, DOP, DiNP, DiDP	vino		extracción liquido-liquido	Synergi Hydro-RP HPLC columna con (2 mm, 4µm, 80A) 10 mM acetato amónico/ metanol, HPLC–MS/MS		1.6-26.6 µg/l	95-105%	(Hayasaka 2014)
Ftalatos:DMeP, DMP, DMEP, DEEP,DEP, DAP, DIPrP, DPrP, DPhP, DBP, DIBP, DBEP,DBeP,BBP, DBuP, DIPP, DPP,DCHP, BMPP, DHXP, DHP,DEeP, DINP, DNP,DIDP, (DNOP) and bis(2-DEHP.	leche y derivados		QUEChERS	ESI (+) (-), UHPLC/ESI Q-Orbitrap)	0.32-2.6 µg/kg		90,7-104.6%	(Jia et al. 2014)

Compuestos	Alimento	Material en contacto	Extracción	LC condiciones (Columna/Fuente de ionización y técnica)	LD	LC	Recuperación	Referencias
Fotoiniciadores y tintas: 3,90_217.1080, 4,62_285,1315, 5,35_251,1260, 5,44_453.1770, 5,50_473.1448, 5,71_337,1627, 6,50_273.2067, 6,50_259.1911, 6,82_389.1118, 6,97_403.2334, 7,05_287,2230, 7,19_297.2412, 7,22_341.2655, 7,23_385,2928, 7,36_315.2549, 7,39_315.2549, 7,62_343.2855, 7,80_371.3174		material multicapa	con simulantes etanol 95% y Tenax	C18 (1.7µmx100x2.1mm) agua 0.1% fórmico/ metanol 0.1% fórmico), ESCI, UPLC-QTOF-Ms				(Aznar et al. 2015)

Sustancias añadidas de forma no intencionada (NIAs): Polímero de formaldehido (PET) , polímero de acetaldehído (PET), etileno tereftalato dímeros y trímeros Polímero (PET), 2,4-Di-tertbutyl-phenol (2,4-DBTP) Polímero (pp) ,2,6-Di-tertbutyl-p-benzoquinone (2,6-DTBQ) Polímero (PP), 3,5-Ditertbutyl-4- ácido hidroxifenilpropionico Polímero (PP), 2,6-Ditertbutyl-4-metoxifenol Polímero (PP), 3,5-Ditertbutil-4-hidroxibenzoic acido Polímero (PP), Trifenilfosfano Polímero (PP), Tri-o-tolylfosfato Polímero (PP) , Difenil fosfato Polímero (PP), Dimetillbenzaldehido Polímero (PP), 4-Hidroxi-1H-indole-3-carboxilico acido Polímero (PP), 7,9-Di-tert-butil-1-oxaspiro(4,5)deca-6,9-dieno-2,8-dione Polímero (PP), Metil-3-(3,5-ditertbutil-4-hidroxifenill)propionato Polímero (PP), 3-[3,5-Di-tert-butil-4-hidroxibenzil]ácido propinoico polímero (PP), Carbonilo, especies vinílicas Polímero (PE), (Z)-9-Octadecenamida Polímero (PE), 2,4-Dit-butil-6-nitro-pfenol Polímero (PE), 2,4-Dit-butil-6-nitro-fenol y 2-cyclohexano-1-diona, 3,5-dimetil, o-metiloxima Polímero (PE) ,Nonilfenol (NP)		polímeros: PET, PRP, PE, materiales impresos, envases activos, adhesivos, latas, PVC	espacio de cabeza, micro extracción en fase sólida, extracción Liquido-Liquido, Micro extracción Liquido-liquido	volátiles GC-MS(QTOF), no volátiles LC-MS(QTOF), ESI				(Nerin et al. 2013)

Compuestos	Alimento	Material en contacto	Extracción	LC condiciones (Columna/Fuente de ionización y técnica)	LD	LC	Recuperación	Referencias
Polímero (PET), Octilfenol (OP) Polímero (PET),aminas aromáticas primarias (PAAS),N2-dodecanoil-l-arginina (LAS) ,C10H16O2 envasado activo, 1,4,7-Trioxaciclotridecano-8,13-diona Adhesivos, derivados de BADGE , ESBO clorhídrinas PVC, Acido abietico y derivados Adhesivos, 1-Hexanol-2-etill Adhesivos, 2-Etilhexilacetato Adhesivos, lactona cíclica Adhesivos, Nonilfenol etoxilado								
Aditivos: BHT, BHA, TNV 234, TNV 326, TNV 327, TNV328, Cys UV 9, Cys UV12, Cys UV 24, Cys UV 5411, I-168, Adv-800, UV-400, Cyx-2246, Ch-81,Uv-OB, I-1076, I-1010, I-1330, I-1081		policarbonato	extracción liquido liquido con cloroformo y metanol	C18 (2,0µmx150x0,3mm) ACN: agua/0,5mMacetato sódico 8,5mM acido acético, UHPLC-HRMS, ESI(+)(-)			95-98%	(Bignardi et al. 2014)

1.2.1. Preparación de muestras

Algunas técnicas convencionales como el Soxhlet, extracción sólido-líquido (S–L), extracción en fase sólida (SPE), extracción por ultrasonidos y la extracción líquido-líquida (L-L) se han utilizado de forma general para la extracción de contaminantes de los envases, así como de los propios alimentos, cómo puede observarse en las figuras 3 y 4.

Soxhlet y SPE se han utilizado para extraer los ftalatos de los materiales de papel y bebidas, posteriormente analizadas por cromatografía de gases (Cinelli et al. 2014, Aznar et al. 2012). Para los compuestos más polares, analizados por cromatografía líquida, la extracción S-L se ha utilizado para extraer BPA y BADGEs de alimentos enlatados (Alabi et al. 2014).

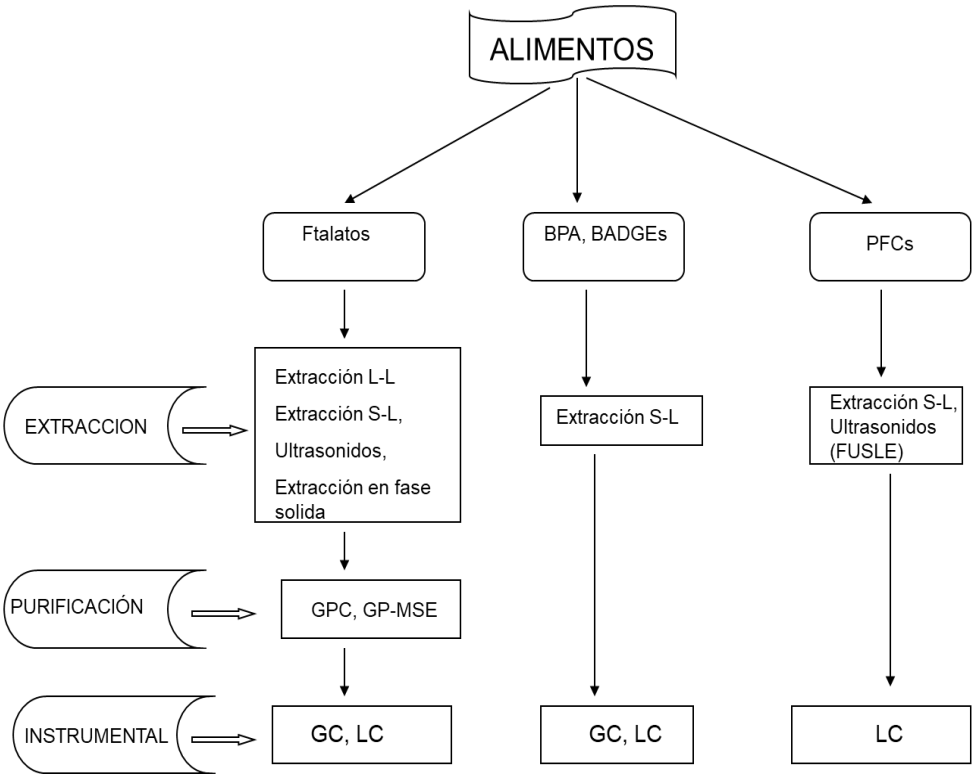


Figura 3. Metodologías analíticas para la determinación de contaminantes en alimentos.

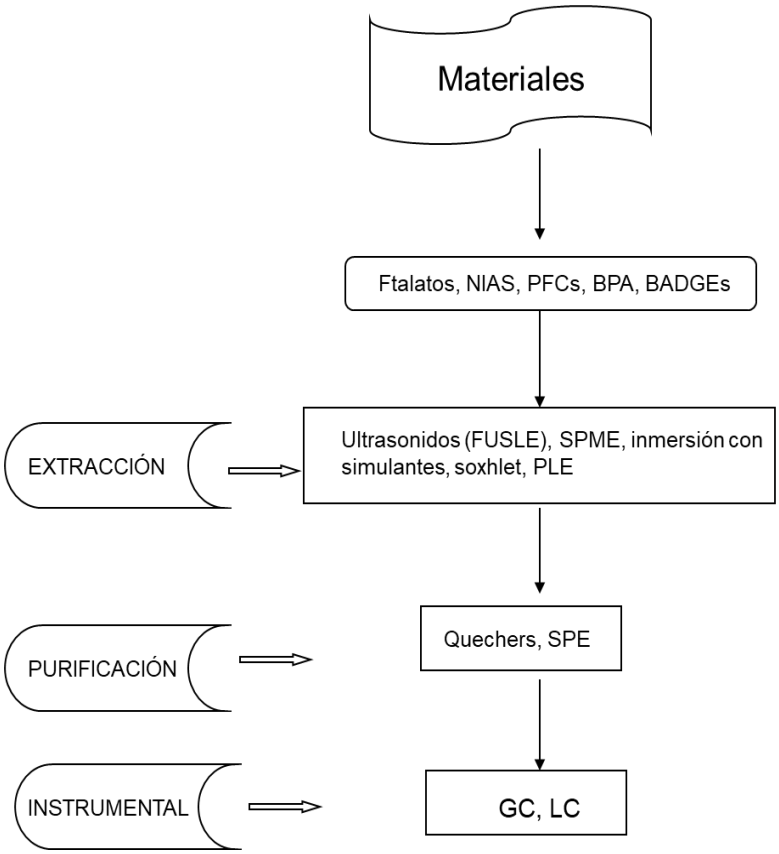


Figura 4. Metodologías analíticas para la determinación de contaminantes en envases.

Otro método de extracción convencional es la extracción por ultrasonidos. Se ha empleado en la preparación de la muestra de ftalatos en alimentos (leche y vino) y del material plástico (Cinelli et al. 2013, Yan et al. 2011). También ha sido utilizada en la preparación de la muestra para la determinación de PFCs de los materiales de papel (Vavrouš et al. 2016).

La cromatografía de permeabilidad en gel (GPC) y micro jeringa de purga de gas (GP-MSE) se utilizaron como purificación, después de la extracción L-L y S-L para la determinación de ftalatos en alimentos como frutas, leche, bebidas, alimentos para bebés, etc. (Fierens et al. 2012, Moreira et al. 2015).

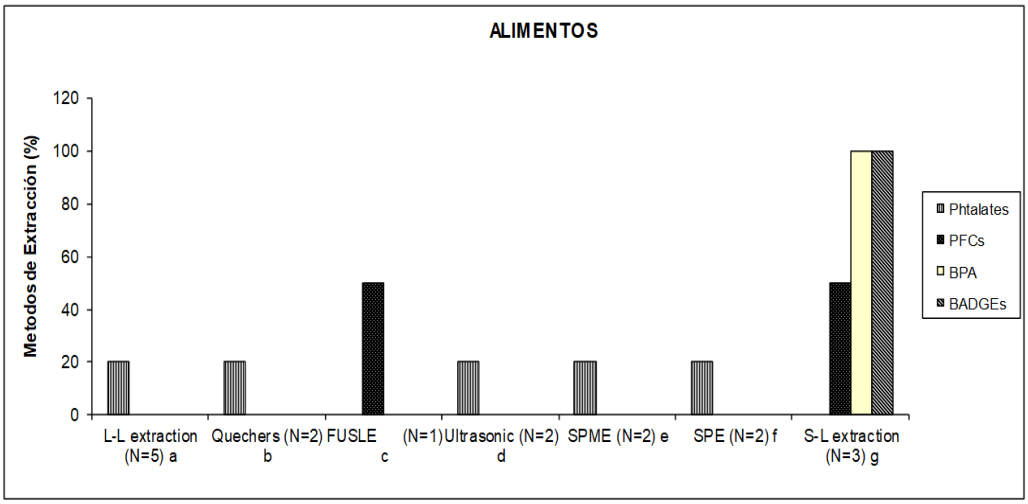
Además, el SPE se ha utilizado como etapa de purificación (clean-up) en el análisis de PFCs en diferentes materiales de papel (papel de horno, bolsas de papel), después de la extracción por ultrasonidos y seguido de cromatografía líquida (Vavrouš et al. 2016).

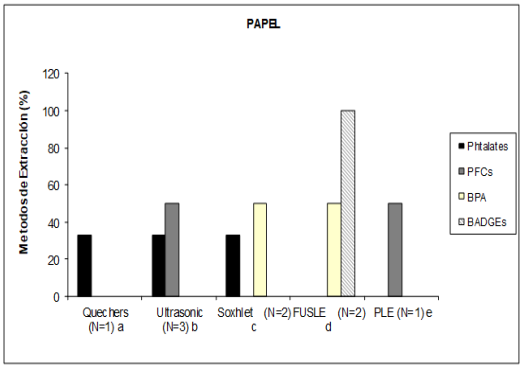
Las principales desventajas de las técnicas convencionales son que la preparación de la muestra es generalmente manual y se requiere una gran cantidad de disolvente orgánico.

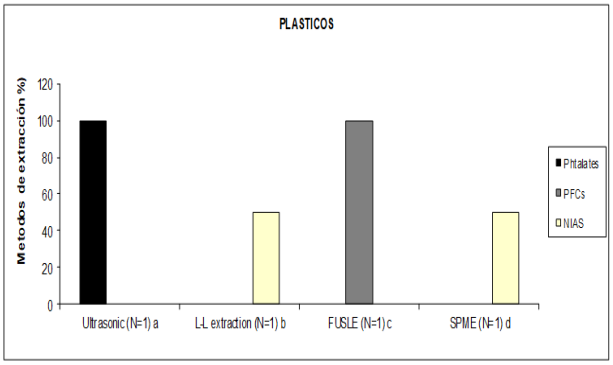
Una etapa de purificación suele ser necesaria cuando se aplican este tipo de técnicas de extracción convencional.

Además de las técnicas convencionales descritas, actualmente se utilizan otras técnicas que han tenido amplia aceptación en los campos ambientales y alimentarios. Entre estos destacan la Microextracción en fase sólida (SPME) (Pawliszyn 2012, Moreira et al. 2015), la extracción sólido–líquido con ultrasonidos (FUSLE) (Picó 2013), la extracción mediante fluidos presurizados (PLE) (Vazquez-Roig et al. 2015) y el método QuEChERS (Anastassiades et al. 2003). Estas técnicas cumplen con el objetivo de minimizar el uso de disolventes orgánicos y evitar en la medida de lo posible la purificación. Estas nuevas metodologías de preparación de muestra se utilizan actualmente para la extracción tanto en materiales como en alimentos.

La figura 5 presenta el porcentaje de utilización de las metodologías de extracción en alimentos y MCA (papel y plásticos) para ftalatos, PFC, BPA, BADGEs y NIAS en los métodos descritos en la bibliografía reciente.







N: Número de publicaciones por cada técnica de etracción; **Alimentos**: a:(Dugo et al. 2011, Fierens et al. 2012, Ostrovsky et al. 2011,Makkliang et al. 2015, Xu Liang Cao 2010), b: (Vavrous et al. 2016, Jia et al. 2014), c: (Moreta et al. 2014), d: (Cinelli et al. 2013, Yan et al. 2011), e: (Rios et al. 2010, Moreira et al. 2015), f :(Cinelli et al. 2014, Xu et al. 2014), g:(Alabi et al. 2014, Zafeiraki et al. 2016, Moreta et al. 2014), **Papel**: a: (Vavrous et al. 2016), b:(Vavrous et al. 2016, Fierens et al. 2012,Ning et al. 2011), c:(Ning et al. 2011,Aznar et al. 2012), d: (Pérez-Palacios et al. 2012, Moreta et al. 2014) e:(Zafeiraki et al. 2014), **Plástico**: a:(Fierens et al. 2012), b:(Nerín et al. 2013), c:(Moreta et al. 2014), d:(Nerín et al. 2013).

Figura 5. Metodologías de extracción en alimentos, papel y plásticos

La SPME se aplica comúnmente en el modo espacio de cabeza (HS-SPME), en general para compuestos volátiles. Aquí la fibra utilizada para la extracción no entra en contacto directo con la muestra. La SPME también se puede aplicar en modo de inmersión directa (DI-SPME), en la que la fibra de extracción se coloca en contacto directo con la muestra, aumentando la eficiencia.

Recientemente, para la determinación de ocho plastificantes (ftalatos), se utilizó la micro extracción en fase sólida con fibra enfriada por corriente de nitrógeno (CF-SPME), seguida de cromatografía de gases, en especias y pollo asado y almacenado en bolsas de

plástico (Moreira et al. 2015). Además, SPME ha sido la técnica seleccionada para la extracción de NIAS de plásticos y de latas (Nerin et al. 2013). La principal ventaja de este tipo de técnicas es la de evitar la manipulación de la muestra minimizando la contaminación del material, disolventes y muestras. Sin embargo, la desventaja de trabajar con fibras es su fragilidad y alto coste.

La técnica FUSLE es una metodología sencilla, segura y económica además de mucho más eficiente que el sistema de extracción ultrasónica tradicional (con baño) (Picó 2013).

Esta técnica se ha utilizado recientemente para la extracción de disruptores endocrinos, tipo Bisfenol en contacto con alimentos, materiales de papel reciclado (Pérez-Palacios et al. 2012) y para los ácidos alquil perfluorados en palomitas y envases de palomitas de maíz para microondas (Moreta et al. 2014). Los factores que influyen en la eficiencia de la extracción FUSLE (volumen del solvente, tiempo de extracción…) fueron optimizados en estos estudios. También BPA, BPF, BADGE y BFDGE, fueron extraídos con 20 mL de metanol en el ultrasonido durante 5 segundos y 2 ciclos de extracción (Pérez-Palacios et al. 2012d).

En otro estudio, ácidos alquílicos perfluorados (PFAA) (nueve ácidos perfluorocarboxílicos (PFCAs) y perfluorooctano sulfonato (PFOS)) fueron extraídos por FUSLE en un solo ciclo de 10 segundos de bolsas de palomitas de maíz para microondas y de palomitas de maíz antes y después de cocinar (Moreta et al. 2014). Se obtuvieron mejores recuperaciones al extraer BPA, BPF, BADGE y BFDGE de papel reciclado (72–97%) (Pérez-Palacios et al. 2012), que al extraer PFC en maíz y palomitas de maíz (65-105%) (Moreta et al. 2014), utilizando el método de extracción FUSLE.

La PLE también se ha utilizado en el tratamiento de muestra de BPA y compuestos relacionados, y en fotoiniciadores, siempre seguido por determinación con cromatografía líquida (Gallart-Ayala et al. 2013). Esta técnica se ha utilizado recientemente para la extracción de compuestos perfluorados (PFCs) utilizados en los materiales en contacto con alimentos (papel de horno y vasos de bebida) como capas para favorecer la resistencia a la humedad. Después de una extracción PLE seguida de una purificación con SPE (con 1,5 g de Florisil, 1 g de alúmina básica y 1 g de sodio), se inyectó con LC – MS/MS. El método de extracción con purificación SPE obtiene recuperaciones entre 60 y 90% (Zafeiraki et al. 2014).

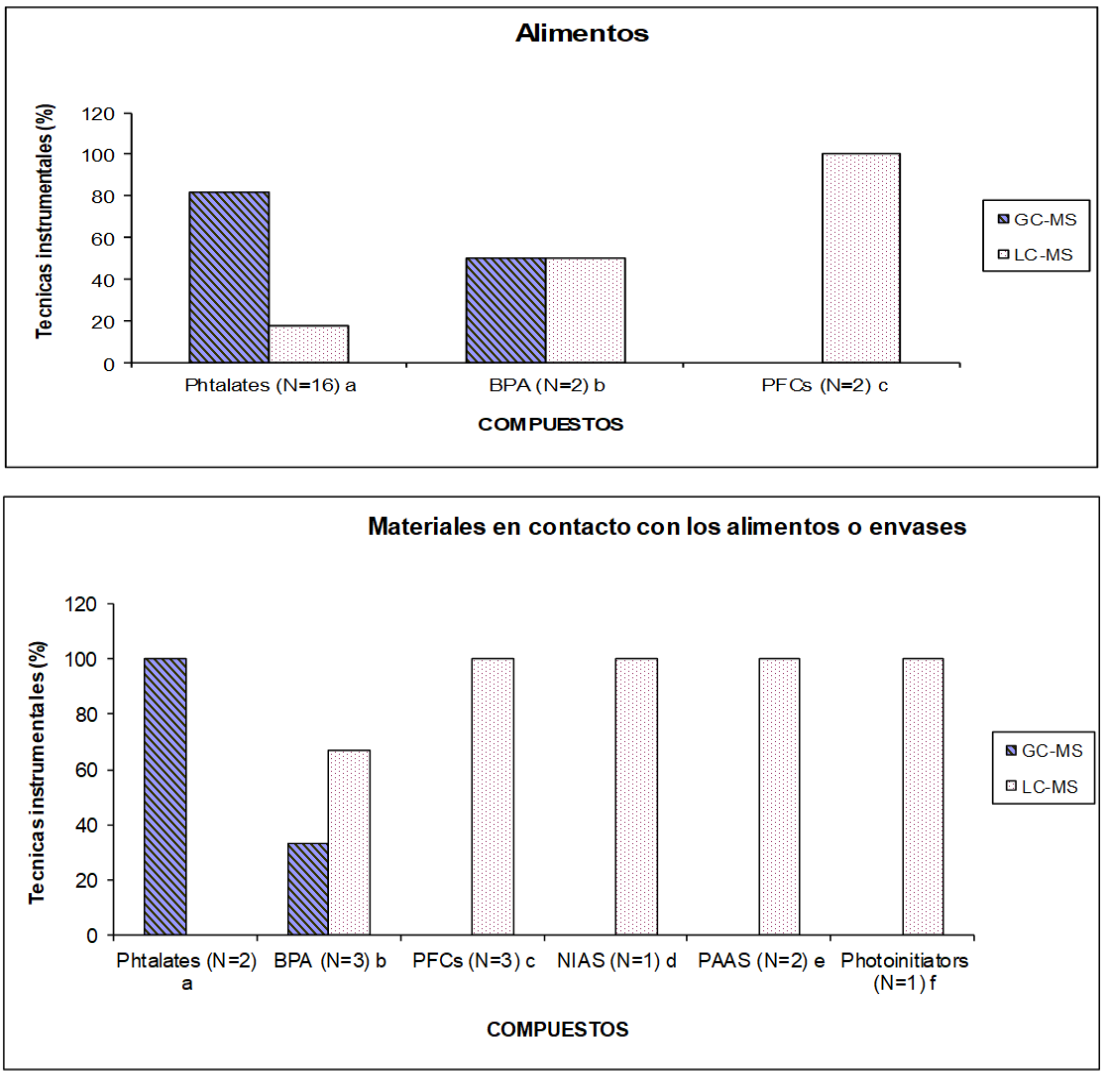
El método QuEChERS fue propuesto por Anastassiades et al. en 2003 (Anastassiades et al. 2003). Se usó por primera vez para extraer pesticidas de los alimentos. Hoy en día, el método QuEChERS se aplica cada vez más a la extracción de otros compuestos. En 2014, permitió extraer 23 ésteres de ftalatos (PAEs) de la uva, especias, fideos de huevo y zumo de pomelo (Ning et al. 2011). En 2016, QuEChERS fue aplicado después de la extracción por ultrasonidos, usando una mezcla de acetonitrilo y agua, para la determinación de 68 contaminantes: ftalatos, hidrocarburos aromáticos policíclicos, fotoiniciadores, bisfenoles y compuestos fluorados en papel (Vavrouš et al. 2016). QuEChERS se ha utilizado en materiales en contacto con alimentos como una técnica de extracción y purificación, obteniendo recuperaciones entre (75 – 115%). Como técnica de extracción en muestras de alimentos para los ftalatos y como una técnica de purificación para PFC en materiales como el papel (70 – 120%) (Vavrouš et al. 2016), seguido en ambos casos de cromatografía líquida. Sin embargo, al extraer los ftalatos de materiales como el papel usando posteriormente la cromatografía de gases, las recuperaciones fueron deficientes (52 – 135%) (Vavrouš et al. 2016).

1.2.2. Técnicas instrumentales

La selección de la técnica instrumental depende de las propiedades fisicoquímicas de las sustancias y su concentración. Para el análisis de contaminantes orgánicos de los materiales en contacto con los alimentos, tanto la GC como lo LC acoplados a detectores de espectrometría de masas (Q, QqQ, Orbitrap), han sido muy utilizados. La cromatografía de gases es la técnica más adecuada para compuestos apolares y volátiles (p.ej., ftalatos). La cromatografía líquida es apropiada para compuestos polares con menos volatilidad o menor estabilidad térmica, tales como: PFC, PAAS, NIAS y Fotoiniciadores.

Las tablas 11 y 12 resumen los estudios más recientes para la determinación de distintas sustancias en las que se ha empleado GC y LC, respectivamente, tanto en alimentos como en materiales (envases utensilios).

Los ftalatos y el BPA son las familias de compuestos orgánicos analizados principalmente por GC (figura 6). Los métodos publicados recientemente determinan un limitado número de compuestos, entre 1 y 25. Esto contrasta claramente con otros campos como el agua, en los que los métodos multiresiduo/multiclase se diseñan para más de 150 sustancias (Yusa et al. 2015).



N: Número de publicaciones por cada técnica instrumental. **Alimentos**: a:(Dugo et al. 2011, Fierens et al. 2012, Zhu et al. 2013, Cinelli et al. 2013, Cinelli et al. 2014, Rios et al. 2010, He et al. 2010, Cacho et al. 2012, Ostrovsky et al. 2011, Moreira et al. 2015, Yan et al. 2011, Makkliang et al. 2015, Xu Liang Cao 2010, Jia et al. 2014, Xu et al. 2014, Hayasaka 2014), b:(Alabi et al. 2014, Aznar et al. 2012), c: (Zafeiraki et al. 2016, Moreta et al. 2014), **Materiales:** a:(Vavrous et al. 2016, Fierens et al. 2012), b:(Perez-Palacios et al. 2012,Aznar et al. 2012, Suciu et al. 2013), c:(Vavrous et al. 2016, Zafeiraki et al. 2016, Moreta et al. 2014), d:(Nerín et al. 2013), e:(Sanchis et al. 2015, Mattarozzi et al 2013), f:(Aznar et al. 2015)

Figura 6. Técnicas instrumentales para alimentos y materiales

Los PAEs se han detectado en aceite (Dugo et al. 2011, Rios et al. 2010), leche (Yan et al. 2011), carne (Fierens et al. 2012), bebidas (Huang et al. 2013), vino (Cinelli et al. 2013), grasa (Ostrovský et al. 2011) y en materiales papel (Vavrouš et al. 2016) y plásticos (Zafeiraki et al. 2014) usando GC – MS. La polaridad de los analitos es el parámetro más importante para la selección de la columna analítica. Debido a la polaridad relativamente baja de los ftalatos, una columna apolar (5% fenil-95% Dimethylpolysiloxane) y una columna mixta (50% fenil-50% Dimethylpolysiloxane) es

las que se utilizan con mayor frecuencia (Mezcua et al. 2012). La Tabla 11 muestra métodos típicos de GC para el análisis de ftalatos en alimentos y materiales. La técnica de ionización más utilizada es el impacto electrónico (EI). Puesto que el contenido de ftalatos en las muestras de alimentos están generalmente a niveles ultra traza, son necesarios detectores altamente sensibles para la identificación y cuantificación. En consecuencia, GC – MS, ha sido la principal técnica para determinar ftalatos debido a su alta sensibilidad y especificidad (Dugo et al. 2011, Fierens et al. 2012, Zhu et al. 2013, Cinelli et al. 2013, Cinelli et al. 2014, Rios et al. 2010, He et al. 2010, Cacho et al. 2012, Ostrovský et al. 2011, Moreira et al. 2015, Yan et al. 2011, Makkliang et al. 2015, GB/T 21911-2008).

En materiales como el papel, se utilizó la espectrometría de masas en modo tándem para el análisis de PAEs (GC-MS/MS) (Vavrouš et al. 2016). Se obtuvieron buenas sensibilidades y recuperaciones para los PAEs en muestras de alimentos, entre el 70% en sopa de pollo (Makkliang et al. 2015) y el 118% en sopa de pollo y verduras (Cacho et al. 2012). En materiales, la migración de PAEs desde el cartón, el tetrabrik y los plásticos presentó buenas recuperaciones que oscilan entre 82 y 99% y límites de detección bajos utilizando GC-EI-MS, en modo SIM (Fierens et al. 2012). Sin embargo, en papel, las recuperaciones obtenidas fueron más bajas (52-135%) con la técnica GC-MS / MS (Vavrouš et al. 2016).

El BPA se ha analizado en sal y azúcar y en cartulina mediante cromatografía de gases (Aznar et al. 2012). En todas estas matrices se emplearon una columna de polisilarileno al 95% - polidimetilsiloxano y una fuente de ionización EI. La espectrometría de masas simple cuadrupolo (MS) se utilizó para obtener límites de detección bajos y un LQ entre 0.05-0.064 mg/L en alimentos y envases (Aznar et al. 2012). En GC, aparte de la detección por espectrometría de masas (aplicada a análisis de ftalatos y BPA), se emplearon otros detectores para analizar NIAS en películas de polipropileno. GC-FID proporcionó un buen LD (0.1-1 mg / kg) en estas matrices plásticas.

Por otra parte, la cromatografía líquida se ha aplicado en gran medida para el análisis de sustancias orgánicas térmicamente inestables y baja volatilidad. LC-MS ha sido la técnica utilizada en el análisis de PFC, PAAS y fotoiniciadores en envases, utensilios de plástico y materiales de papel (figura 6). Además, se ha utilizado LC para el análisis de PFCs en alimentos (maíz, palomitas de maíz, huevos), así como de BPA y BADGE en alimentos

enlatados y ftalatos en productos lácteos, bebidas, semillas, carne, aceite, bizcocho, vino y conservas (Alabi et al. 2014, Zafeiraki et al. 2016, Moreta et al. 2014, Ning et al. 2011).

Algunos autores propusieron un método de UHPLC para mejorar la sensibilidad en el análisis de NIAS (Bignardi et al. 2014), ftalatos (Jia et al. 2014), PFCs (Moreta et al. 2014) o tintas (Aznar et al. 2015). Generalmente se usaron LC, que utilizan columnas C18 con tamaños de partícula de 1.7-5 µm (ver Tabla 12) (Mattarozzi et al. 2013b, Aznar et al. 2015, Pérez-Palacios et al. 2012). Sanchis et al. (2015) probaron diferentes columnas para lograr una separación cromatográfica adecuada de las aminas aromáticas primarias individuales (PAAs). La mejor separación se logró en una columna de fenil-hexil que está compuesta por un grupo fenilo unido a éter con un extremo polar (Sanchis et al. 2015).

Con respecto a la ionización en LC, la ionización por electrospray (ESI) es la más utilizada. El modo de ionización positiva se emplea generalmente para analizar PAAS, BADGE y BFDGE, así como, fotoiniciadores y di ésteres de ftalato, mientras que el modo de ionización negativa proporciona mejor sensibilidad para la detección de metabolitos de ftalatos, BPA, y otros bisfenoles (por ejemplo, BPE, BPB, BPF y BPS) así como, PFCs (Tabla 12). En general, el modo ESI negativo y el ESI positivo generan la molécula desprotonada, $[M-H]^-$, o la molécula protonada, $[M+H]^+$, respectivamente. Ocasionalmente puede darse la fragmentación en la fuente, por ejemplo, con algunos fotoiniciadores de tinta UV (HMPP, HCPK, DMPA, DEAB) (Pawliszyn 2012). En algunos casos, también se observó la formación de iones aductos con componentes de la fase móvil. BADGEs y BFDGEs mostraron una alta tendencia a formar aductos tales como $[M + Na]^+$, $[M + K]^+$, $[M + NH4]^+$ y $[M + ACN]^+$ iones, algunos de estos iones (por ejemplo, $[M + Na]^+$) son muy estables y no se produce fragmentación en MS / MS, pero para aductos de amonio en MS / MS sí se produjo fragmentación (Picó 2013, Vazquez-Roig et al. 2015). En estos casos, para permitir la formación de aductos de amonio y asegurar la reproducibilidad de la señal, el tampón de formiato de ácido fórmico/amonio se utiliza como un aditivo en la fase móvil trabajando en modo ESI positivo.

Para conseguir una buena separación cromatográfica y mejorar la sensibilidad del método, es importante seleccionar las fases móviles apropiadas. Debido a las diferencias en las propiedades fisicoquímicas de los PAEs, la elución en gradiente es generalmente necesaria y el metanol-agua con una pequeña cantidad de ácido fórmico o ácido acético sirve comúnmente como fase móvil (Fan et al. 2014, Ning et al. 2011). El acetonitrilo a

menudo sustituye al metanol debido a su menor coeficiente de viscosidad y mayor capacidad de elución (Cao 2010). Se obtuvieron buenas recuperaciones (75-115%) utilizando metanol al 0,1% de ácido fórmico para analizar las PAEs en alimentos con un LD de 0,8-15 µg / kg en LC-MS / MS (Ning et al. 2011).

Alabi et al (2014), no obtuvieron una resolución de picos satisfactoria para la separación de los 12 bisfenoles y los diglicildímeros seleccionados, así como los isómeros de BFDGE y BFDGE · 2HCl utilizando metanol como disolvente orgánico en la fase móvil. La mezcla de agua y acetonitrilo en condiciones isocráticas mejoró la resolución, pero no fue suficiente para la separación del analito. El uso de la elución de gradiente con una regulación adicional del flujo permitió la separación de la línea de base y una mejor resolución de picos. La mezcla de acetonitrilo / agua para extraer BPA, BADGE y compuestos relacionados en alimentos enlatados utilizando detección por LC - fluorescencia presentó buenas recuperaciones (80-110%) con un LC de 0.9-3.5 µg / kg (Alabi et al. 2014)

Se probaron diferentes fases móviles y flujos para el análisis de PFCs por UHPLC-QTOF-MS / MS en bolsas de maíz y palomitas de maíz (Moreta et al. 2014). En este trabajo, se seleccionó una mezcla de ácido fórmico al 0,8% en ACN y una solución acuosa de ácido fórmico al 0,8% para obtener la mejor separación cromatográfica. También se probaron dos tampones que consistían en ácido fórmico y formiato de amonio o formiato de sodio. Sin embargo, el uso de esos tampones fue descartado. Se seleccionó un flujo de 0,5 ml / min porque flujos más altos empeoraron la resolución cromatográfica. Se obtuvieron mejores recuperaciones en bolsas de palomitas de maíz para microondas (80-106%) que en matrices de alimentos (maíz y palomitas de maíz) (65-105%), aunque se consiguió mejor LQ en alimentos (0.2-0.6 ng g) utilizando un QTOF con detector MS / MS (Moreta et al. 2014).

Mattarozzi et al. (2013) desarrollaron un método para el análisis de 22 PAAS en láminas de plástico por LC-HRMS, con un LQ entre 0.099-5.45 µg / kg. Los autores utilizaron el ácido PFPA como modificador a una concentración de 4.7 mM, asegurando que las aminas estuviesen en forma protonada. La optimización del gradiente metanol / agua con PFPA permitió una buena resolución de todos los analitos, con valores de RSD inferiores al 2%.

Además, en esta tesis, LC-HRMS también se ha utilizado para la determinación de PAAs en utensilios de cocina de nylon (Sanchis et al. 2015). En este caso, se eligió metanol/agua

como fase móvil sin modificadores, logrando un LQ de 2.5 µg / kg y RSD inferiores al 2%.

Debido a las potentes características analíticas del LC-HRMS existe una clara tendencia a su aplicación en este campo, aunque de momento sean escasos los métodos publicados. Esta técnica ofrece la posibilidad de detectar cientos de contaminantes polares y además de ser una técnica cuantitativa, permite el análisis retrospectivo y la detección de sustancias sospechosas ("suspect screening") o la detección de sustancias desconocidas ("non-target analysis") (Yusa et al. 2015, Mezcua et al. 2012). El uso de analizadores de masas de alta resolución como TOF u Orbitrap, para el análisis multiresiduo, se debe principalmente a las ventajas de usar el modo de adquisición en "full scan" (escaneo completo), combinado con un alto poder de resolución (> 50,000 FWHM) y una exactitud de masa < 5ppm (Sanchis et al. 2018, Yusa et al. 2015). Recientemente, se han publicado métodos de LC-HRMS con analizador de masas TOF u Orbitrap para el análisis de fotoiniciadores (Sanchis et al. 2018), PFCs (Moreta et al. 2014), PAAs (Mattarozzi et al. 2013, Sanchis et al. 2015), aditivos (Bignardi et al. 2014) y ftalatos (Jia et al. 2014) en MCAs. Esta técnica ha permitido la identificación de NIAS, como moléculas desconocidas que posiblemente se derivan de la degradación del policarbonato (Bignardi et al. 2014) o ftalatos que posiblemente están presentes en utensilios de cocina de nylon (Sanchis et al. 2015).

1.3. Exposición a contaminantes de MCA

La evaluación de la exposición y del riesgo a contaminantes, es una de las actividades de mayor importancia en el campo de la salud pública, que requiere de un esfuerzo de investigación conjunto en distintos campos como la toxicología, la química analítica o la epidemiología.

La evaluación de la exposición, así como la evaluación del riesgo a contaminantes procedentes de los materiales en contacto con los alimentos (bisfenoles, ftalatos, aminas) se realiza mediante la evaluación de la exposición externa o control ambiental, determinando los niveles de contaminantes en los distintos medios como aire o alimentos, y combinándola con los datos de ingesta o inhalación. Por otra parte, la exposición interna, biomonitorización en humanos, se evalúa midiendo los contaminantes en fluidos biológicos y también se compara con los valores de referencia para evaluar el riesgo. Un esquema de estos dos enfoques se detalla en la figura 7.

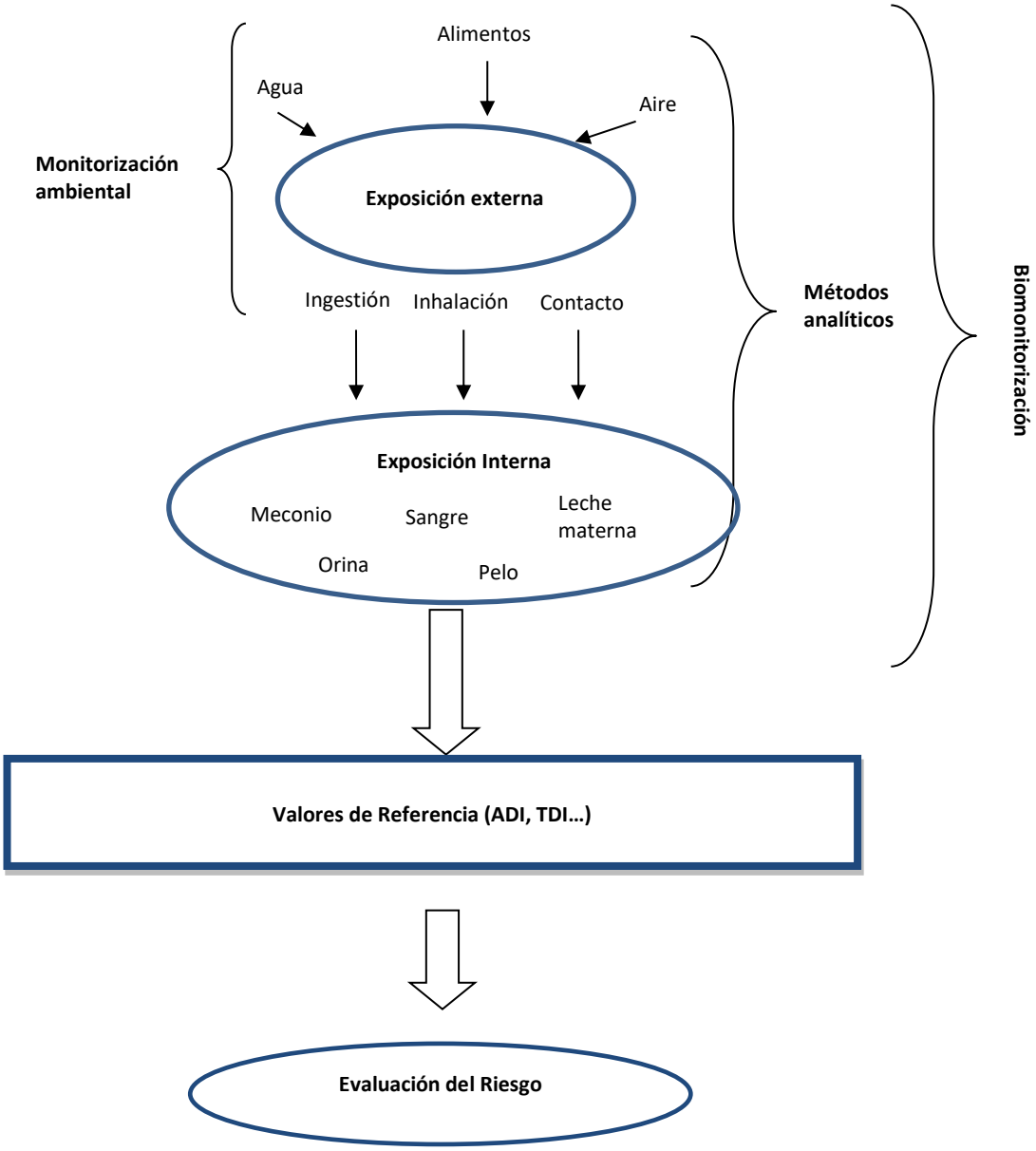


Figura 7. Estrategias para la evaluación de la exposición a contaminantes

Exposición externa

La presencia de un contaminante en el medio ambiente (agua, aire y alimentos) que rodea un individuo puede originar la exposición de éste al contaminante. La consecuencia de esta exposición es que cierta cantidad del contaminante podrá alcanzar o incorporarse al organismo del individuo, produciendo determinados efectos sobre la salud de éste. Mediante la metodología de la exposición externa, se estima la exposición midiendo los niveles de contaminantes en alimentos, agua o aire y combinándolos con la ingesta o inhalación de estas matrices. No obstante, ambos factores tienen interés propio, por lo

cual se dice que la exposición es más o menos intensa según sea la magnitud de la concentración del contaminante y la cantidad o nivel de ingesta del alimento que lo contiene (Bartual et al. 2016). Los alimentos constituyen la principal ruta de entrada en el cuerpo humano de gran parte de los contaminantes presentes en los MCA y que pueden migrar a los alimentos. Estudios como los de dieta total para contaminantes específicos y la monitorización de ftalatos y bisfenoles o derivados epóxidos procedentes del plástico (Zhu et al. 2013 , Cacho et al. 2012, Yang et al. 2015, Gallart-Ayala et al. 2011), posibilitan el conocimiento de la contaminación de los alimentos y la ingesta en una población determinada. Mediante la comparación con los estándares toxicológicos (ingesta diaria admisible IDA, ingesta diaria tolerable TDI, etc.) es posible llevar a cabo una evaluación de la exposición y una caracterización del riesgo (Marín 2014).

Exposición interna

Un segundo enfoque para evaluar la exposición es la biomonitorización en humanos (exposición interna) que consiste en una medida de los contaminantes en los fluidos biológicos. La cantidad del contaminante que incorpora el individuo constituye la dosis absorbida o dosis interna. La cantidad de tóxico que, como consecuencia de aquella dosis, alcance un determinado compartimento u órgano del cuerpo del individuo constituirá la dosis local recibida por el mismo y será la causante de los efectos del tóxico (Beser et al. 2019, Marín et al. 2018, Pérez et al. 2017).

La biomonitorización en humanos de contaminantes procedentes de materiales plásticos o envases ha sido estudiada particularmente para ftalatos y bisfenoles en los últimos años (Arbuckle et al. 2015, Dereumeaux et al. 2016, Myridakis et al. 2015, Vandentorren et al. 2011).

Para la correcta evaluación de la exposición interna, la biomonitorización en humanos es una herramienta de gran utilidad. Se realizan mediante una sistemática y protocolizada recogida de muestras biológicas que permiten el análisis de las concentraciones de los contaminantes y/o sus metabolitos (biomarcadores de exposición), así como, la comparación de los niveles observados con valores de referencia basados en salud para obtener una mejor valoración de la exposición interna y una completa evaluación del riesgo (Barr et al. 2005, Pérez et al. 2017, Marín et al. 2018).

Es indispensable seleccionar tanto la matriz biológica como los biomarcadores más adecuados para cada tipo de contaminante, así como disponer de métodos analíticos

fiables, aplicados bajo estrictos protocolos de calidad, además de disponer de referencias para la interpretación de los resultados (Angerer et al. 2007, Pérez et al. 2017).

La sangre (plasma, suero) es la matriz idónea para muchos contaminantes persistentes lipofílicos como los pesticidas organoclorados (aldrin, dieldrin, clordano...), difenilos polibromados (PBDE's) o policlorados (PCB´s), ya que está en contacto con todos los tejidos y con los órganos donde se pueden depositar estos compuestos (Yusa et al. 2015). Sin embargo, se trata de una técnica invasiva.

Por otro lado, la orina se considera una matriz de gran interés para el análisis de contaminantes no persistentes, contaminantes hidrófilos que se excretan rápidamente como los fenoles, metabolitos de los ftatalos (Fromme et al. 2007, Beser et al. 2019) o de los insecticidas piretroides (Yusa et al. 2015). La orina es fácil de recoger y no es una técnica invasiva. El desarrollo de nuevas metodologías analíticas está permitiendo el uso de otras matrices como pelo, uñas, saliva y leche materna, sin embargo, son necesarios más estudios para establecer correlaciones entre las concentraciones en las matrices y carga total en el cuerpo (Esteban et al. 2009)

Los programas de biomonitorización y la mayoría de los estudios utilizan fundamentalmente biomarcadores de exposición entre los que se encuentran aquellos contaminantes o sus metabolitos a los que se presta una mayor atención para evaluar sus dosis internas tales como: fenoles, metales (Pérez et al. 2019, Roca et al. 2016), plaguicidas (Yusa et al. 2015), hidrocarburos policíclicos aromáticos (PAH's), dioxinas, difenilos polibromados (PBDE's), compuestos perfluorados y ftalatos entre otros.

En la presente Tesis Doctoral se aborda la puesta a punto de nuevas metodologías analíticas para la investigación de algunos grupos de contaminantes procedentes de MCA en varias matrices que permiten la evaluación tanto de la exposición interna como externa. Referente a la exposición externa se han estudiado diferentes familias de compuestos: aminas, fotoiniciadores, BADGEs y PFCs en simulantes de distintos tipos de alimentos y en plásticos y otras sustancias no deseables (NIAS) en simulantes y materiales. Por otro lado, respecto a la exposición interna se han realizado estudios para biomonitorización en orina humana de contaminantes poco estudiados hasta el momento, como lo son los bisfenoles F y S además del bisfenol A que ya ha sido más estudiado, y los parabenos.

1.4. Artículo 1. *Analytical strategies for organic food packaging contaminants*

Journal of Chromatography A, 1490 (2017) 22–46

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Review article

Analytical strategies for organic food packaging contaminants

Yovana Sanchis[a,b], Vicent Yusà[a,b,c,*], Clara Coscollà[a,b]

[a] Foundation for the Promotion of the Health and Biomedical Research in the Valencia Region (FISABIO-Public Health), 21, Avenida Catalunya, 46020 Valencia, Spain
[b] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain
[c] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form 12 January 2017
Accepted 26 January 2017
Available online 1 February 2017

Keywords:
Food contact material contaminants
Liquid chromatography (LC)
Gas chromatography (GC)
Mass spectrometry (MS)
High resolution mass spectrometry (HRMS)

ABSTRACT

In this review, we present current approaches in the analysis of food-packaging contaminants. Gas and liquid chromatography coupled to mass spectrometry detection have been widely used in the analysis of some relevant families of these compounds such as primary aromatic amines, bisphenol A, bisphenol A diglycidyl ether and related compounds, UV-ink photoinitiators, perfluorinated compounds, phthalates and non-intentionally added substances.

Main applications for sample treatment and different types of food-contact material migration studies have been also discussed. Pressurized Liquid Extraction, Solid-Phase Microextraction, Focused Ultrasound Solid-Liquid Extraction and Quechers have been mainly used in the extraction of food contact material (FCM) contaminants, due to the trend of minimising solvent consumption, automatization of sample preparation and integration of extraction and clean-up steps.

Recent advances in analytical methodologies have allowed unequivocal identification and confirmation of these contaminants using Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC–HRMS) through mass accuracy and isotopic pattern applying. LC–HRMS has been used in the target analysis of primary aromatic amines in different plastic materials, but few studies have been carried out applying this technique in post-target and non-target analysis of FCM contaminants.

Contents

* Corresponding author at: Foundation for the Promotion of the Health and Biomedical Research in the Valencia Region (FISABIO–Public Health), 21, Avenida Catalunya, 46020 Valencia, Spain.
E-mail address: yusa_vic@gva.es (V. Yusà).

http://dx.doi.org/10.1016/j.chroma.2017.01.076
0021-9673/

1. Introduction

Food contact materials are all materials and articles intended to come into contact with food, such as packaging and containers, kitchen equipment, cutlery and dishes. Modern food packaging materials are designed to fulfil multiple purposes including the protection of food products from external sources of contamination and damage, and the information of consumers about ingredients and nutritional data [1]. Furthermore, food packaging provides preservation, ease of transportation and storage of food products [2,3]. Thus, the production and use of packaging materials has increased during the last decades, comprising an indispensable part of food manufacturing. The packaging manufacturing industry is making an effort to combine low cost manufacturing with appearance improvement for consumer appeal, maintenance of food safety and a minimal environmental impact. Concerning food safety, one of the major considerations of manufacturers must be the migration of harmful chemical compounds from packaging materials to food, as they could adversely affect consumer health [4]. In this context, strict national and international regulations, applicable to all materials which come in direct contact with food, have been established. In a European Framework, Regulation 1935/2004/EC [5] regulates materials and articles intended to come into contact with food, providing the basis for securiting a high level of protection of human health. In addition, Regulation 2023/2006 [6] describes the good manufacturing practices (GMP) that industry should follow to protect the interest of consumers.

The majority of studies published in the literature are mostly focused on the analysis of FCM contaminants in materials rather than in food matrices and food simulants. Fig. 1 shows that non-intentionally added substances (NIAS), primary aromatic amines (PAAS) and inks (photoinitiators) have only been analyzed in food contact materials [7–11]. Although contaminants such as perfluorinated compounds (PFCs) and bisphenol A (BPA) have been mainly determined in materials [12–14], these contaminants have also been determined in food, in some studies [15,16]. Instead, phthalates have been more frequently studied in food [17–28,29,30] (see Fig. 1). NIAS, BPA, phthalates, PAAS and inks have been analyzed in food simulants [7,8,10,11]. Table 1 shows food contact contaminants that could be transferred to the food.

Seventeen different materials such as ceramics, cork, rubber, glass, plastics, metals, paper and board, silicones, wood and so on, are described in the Regulation 1935/2004/EC [5] to be in contact with food materials. In the present study, we have focused our work mainly on plastic materials, due to plastic is one of the main material used and, consequently, analyzed in FCM studies. Plastic materials are composed by monomers and other starting substances transformed through chemical reactions in a polymer, which represents the principal component. The most widely produced synthetic plastic polymers are poly vinyl chloride (PVC), polyvinyl acetate (PVA), polyethylene (PE) and polypropylenes (PP), in which polymerization is made by monomers of vinyl chloride, vinyl acetate or simple alkenes (ethane and propene), respectively. Between the most widely used plastic materials are kitchen equipment such as nylon utensils, plastic laminates (plastic films) and other enamelware made of polycarbonates [7,8]. Polycarbonates (PC) are one of the high-performance heterochain polymeric materials that comprise the family of engineering thermoplastics with a wide variety of applications due to excellent mechanical properties, high impact strength, heat resistance and high modulus of elasticity, as well as excellent toughness, clarity and transparency. These properties make it an ideal choice for tableware, microwave ovenware, reusable bottles, food storage containers and water pipes [30,35]. European Regulation 10/2011 is based on a positive list of authorized substances which may be intentionally used in the manufacture of plastic layers in plastic materials and articles and related restrictions, such as specific migration limits (SML). SML means the maximum permitted amount of a given substance released from a material or article in to food or food simulants [36].

Other commonly used materials are paper and paperboard. They are used in corrugated boxes, wrapping paper, milk cartons, folding cartons, bags and sacks, paper plates and beverage cups, fast-food containers, microwave popcorn bags, ice cream cups, dessert containers, baking paper, etc. More specifically, paperboard, due to its thickness, is commonly used for shipping-packages, such as boxes and cartons, but also as packaging material for fast food, such as pizza. Plain paper does not possess good heat-sealing and barrier properties, so it is almost always treated, coated, or impregnated with additives in order to improve its functional and protective properties, before its use as packaging material [37]. In addition, recycled paper is mainly used in direct contact with dry foodstuff like flour, grain, sugar, salt, rice and pasta. A large number of chemicals are used in the paper recycling process, such as bleach, paper strengthening agents and inks. Cardboard packaging materials are mainly contaminated with mineral oil via the fibre recyclate stream [34]. There is an increasing public demand for avoiding the use of waste paper, as packaging material that comes into contact with food products. This demand can be attributed to the shortage of available data concerning migration models of the referred contaminants, but also due to the lack of specific laws for paper and paperboard food packaging. The European regulation (Reg. CE 1935/2004) [5] only affirms that recycled paper can be used for solid, dry foods. To date European laws have not yet provided formal guidelines for reclaimed-fibre testing to assess fibre risk, despite suggesting an approach similar to that for plastics.

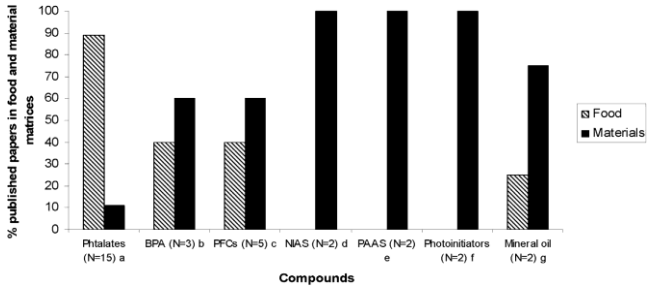


Fig. 1. Publications in food and materials for some food packaging contaminants. (*N* = number of papers published for each compound; Letters = references). a: [12,17,18,21,22,23,24,25,26,27,28,29,33,101,102]; b: [15,30,31]; c: [12,13,16,32,96]; d: [9]; e: [7,8]; f: [10,12], g [98,99].

Table 1
Food contact material contaminants, food products and food contact materials.

Compound	CAS number	Abbreviation	Food product	Food contact material	Reference
Primary aromatic amines (PAAS)					
Aniline	62-53-3	ANL		Plastic (nylon kitchen utensils)	[8]
2,4 Dimaminetluene	95-80-7	2,4 TDA		Plastic (nylon kitchen utensils)	[8]
Benzidine	92-87-5	BNZ		Plastic (plastic laminate)	[7]
o-Ansidine	90-04-0	o-ANS		Plastic (plastic laminate)	[7]
4,4′ Diaminodiphenylether	101-80-4	4,4′ DPE		Plastic (nylon kitchen utensils)	[8]
o-Toludine	95-53-4	o-TOL		Plastic (plastic laminate)	[7]
4,4′ Methylenedianiline	101-77-9	4,4′ MDA		Plastic (nylon kitchen utensils)	[8]
o-Diansidine	119-90-4	o-DANS		Plastic (plastic laminate)	[7]
o-Tolidine	119-93-7	o-TOLI		Plastic (nylon kitchen utensils)	[8]
p-Chloroaniline	106-47-8	p-ANL		Plastic (plastic laminate)	[7]
p-Cresidine	120-71-8	p-CRS		Plastic (plastic laminate)	[7]
4,4′ Methylene-bis-(2-methylaniline)	838-88-0	4,4′ MBM		Plastic (nylon kitchen utensils)	[8]
4,4′ Thiodianiline	139-65-1	4,4′ TDA		Plastic (plastic laminate)	[7]
2-Naphtylamine	91-59-8	2-NAPH		Plastic (plastic laminate)	[7]
4-Chloro-o-toludine	95-69-2	4-Cl-TOL		Plastic (plastic laminate)	[7]
5 Nitro-o-toludine	99-55-8	5-N-O-TOL		Plastic (plastic laminate)	[7]
2,4,5 Trimethylaniline	137-17-7	2,4,5 MTA		Plastic (plastic laminate)	[7]
4-Aminobipheny	92-67-1	4-ABF		Plastic (plastic laminate)	[7]
4,4′ Methylene-bis-(2-chloroaniline)	101-14-4	4,4′ M(2Cl)		Plastic (plastic laminate)	[7]
3,3′ Dichlorobenzidine	91-94-1	3,3′ DCB		Plastic (plastic laminate)	[7]
p-Aminobenzene	60-09-3	p-ABZ		Plastic (plastic laminate)	[7]
m-Phenyldiamine	108-45-2	m-PDA		Plastic (nylon kitchen utensils)	[8]
1,5 Diamine naphtalene	2243-62-1	1,5 DAN		Plastic (nylon kitchen utensils)	[8]
o-Aminoazotoluene	97-57-3	o-AaT		Plastic (plastic laminate)	[7]
BPA, BADGEs and related compounds					
Bisphenol A 2,2-bis(4-hydroxyphenyl)propane	80-05-7	BPA	Canned food		[15]
Bisphenol F bis(4-hydroxyphenyl)METHANE	620-62-8	BPF		Recycled paper	[31]
Bisphenol A diglycidyl ether	1675-54-3	BADGE		Recycled paper	[31]
Bisphenol f diglycidyl ether	2095-03-6	BFDGE		Recycled paper	[31]
Perfluorinated compounds (PFCs)					
Perfluorooctanesulfonic acid tetraethylammonium salt	1763-23-1	PFOS	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluorobutanoic acid	375-22-4	PFBA	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluoropentanoic acid	2706-90-3	PFPeA	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluorohexanoic	307-24-4	PFHxA	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluroheptanoic acid	375-85-9	PFHpA	Popcorn	Plastic (popcorn bags)	[32]
Perfluorooctanoic acid	335-67-1	PFOA	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluorononanoic acid	375-95-1	PFNA	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluorodecanoic acid	335-76-2	PFDA	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluoroundecanoic acid	2058-94-8	PFUnA	Popcorn	Plastic (popcorn bags)	[32]
				Paper	[12]
Perfluorododecanoic acid	307-55-1	PFDoA		Paper	[12]
Perfluoroctylsulfonamide	754-91-6	FOSA		Paper	[12]
Perfluorobutanesulfonic acid	29420-49-3	PFBS		Paper	[12]
Perfluorooctanephosphonic acid	–	PFOPA		Paper	[12]
Perfluorohexanesulphonic acid	355-46-4	PFHxS		Paper	[12]
Perfluorohexanephosphonic acid	–	PFHxPA		Paper	[12]
Perfluorododecanoic acid	307-55-1	PFDoA	Popcorn	Plastic (popcorn bags)	[32]
Perfluorodecanephosphonic acid	–	PFDPA		Paper	[12]
Phthalates					
Dimethyl phtalate	131-11-3	DMP	Olive oils		[17]
		DMP	Food products	Plastics, paperboard	[18]
		DMP	Fatty foods		[19]
		DMP	Hydroalcoholic food beverages		[21]
		DMP	Fatty foods		[22]
		DMP	Wine		[20]
		DMP	Soybean milk		[23]
		DMP	Vegetables		[24]

Table 1 (*Continued*)

Compound	CAS number	Abbreviation	Food product	Food contact material	Reference
Bis (2-methoxyethyl)phtalate	117-82-8	DMEP	Fatty foods		[19]
		DMEP	Fatty foods		[25]
Bis(2-ethoxyethyl)phtalate	605-54-9	DEEP	vegetables		[24]
Diethyl phtalate	84-66-2	DEP		Paper	[12]
		DEP	Cow milk/power milk		[17]
		DEP	Hydroalcoholic food beverages		[21]
		DEP	Fatty foods		[19]
		DEP	Olive oils		[17]
		DEP	Meat roasted		[26]
		DEP		Plastics, paperboard	[18]
		DEP	Vegetables		[24]
Diallyl phtalate	131-17-9	DAP	Fatty foods		[22]
		DAP	Soybean milk		[23]
Diisopropyl phtalate	605-45-8	DIPrP	wine		[20]
		DIPrP	Bottle milk		[27]
Diphenyl phtalate	84-62-8	DPhP	Soybean milk		[23]
		DPhP	Fatty food		[25]
		DPhP		Paper	[12]
		DPhP	Milk and milk products		[33]
Dibutyl phtalate	84-74-2	DBP	Hydroalcoholic food beverages		[21]
		DBP	Milk and milk products		[33]
		DBP	Olive oils		[17]
		DBP	Meat roasted		[26]
		DBP		Paper	[12]
		DBP	Vegetables		[24]
		DBP	Fatty foods		[22]
		DBP	Fatty food		[25]
		DBP	Bottled milk		[27]
		DBP	Chicken soup		[28]
		DBP	Fatty foods		[19]
		DBP	Wine		[20]
		DBP	Soybean milk		[23]
Diisobutylphtalate	84-69-5	DIBP	Vegetables		[24]
		DIBP	Olive oils		[17]
		DIBP		Paper	[12]
		DIBP		Plastics, paperboard	[18]
		DIBP	Fatty foods		[22]
		DIBP	Wine		[20]
		DIBP	Hydroalcoholic food beverages		[21]
		DIBP	Meat roasted		[26]
		DIBP	Fatty foods		[19]
Bis(2-butoxyethyl)phtalate	117-83-9	DBEP	Fatty foods		[19]
		DBEP		Plastic (plastic bags)	[28]
Dibenzyl phtalate	523-31-9	DBeP	Milk/milk products		[33]
Benzylbutylphtalate	85-68-7	BBP	Bottle milk		[27]
		BBP		Plastics, cardboard	[18]
		BBP	Wine		[20]
		BBP	Olive oils		[17]
		BBP	Hydroalcoholic food beverages		[21]
		BBP	Vegetables		[24]
		BBP	Fatty foods		[22]
		BBP	Meat roasted		[26]
Di-n-butylphtalate	84-74-2	DnBP	Vegetables		[24]
Dipentyl phtalate	131-18-0	DPP	Hydroalcoholic food beverages		[21]
Dipentylphtalate			Fatty foods		[22]
Dicyclohexyl phtalate	84-61-7	DCHP	Fatty food		[25]
		DCHP	Fruits and vegetables,milk, cereals meat, fish, fat and oils, snacks, condiments, beverages, and baby food		[18]
		DCHP	Meat samples(roasted chicken)		[26]
		DCHP		Paper	[12]
Dihexyl phtalate	84-75-3	DHXP	Wine		[20]
Diheptylphtalate	3648-21-3	DHP	fatty foods		[22]
		DHP	Meat roasted		[26]
Diisononyl-phtalate	28553-12-0	DINP		Paper	[12]
		DINP	Olive oils		[17]
		DINP	Bottle milk		[27]
		DINP	Fatty foods		[22]

Table 1 (*Continued*)

Compound	CAS number	Abbreviation	Food product	Food contact material	Reference
Dioctyl phtalate, bis(2-ethylhexyl) phtalate	117-81-7	DNOP, DEHP	Meat roasted		[26]
		DNOP, DEHP		Paper	[12]
		DNOP, DEHP	Soybean milk		[23]
		DNOP, DEHP	Fatty foods		[19]
		DNOP, DEHP	Bottle milk		[27]
		DNOP, DEHP		Plastics, cardboard	[18]
		DNOP, DEHP		Plastics, cardboard	[18]
		DNOP, DEHP	Meat samples(roasted chicken)		[26]
		DNOP, DEHP		Paper	[12]
		DNOP, DEHP	Fatty foods		[22]
		DNOP, DEHP	Olive oils		[17]
		DNOP, DEHP	Wine		[20]
		DNOP, DEHP	Vegetables		[24]
		DNOP, DEHP	Fatty foods		[19]
		DNOP, DEHP	Hydroalcoholic food beverages		[21]
Di-n-heptyl phthalate	3648-21-3	DiHP	olive oils		[17]
Di-n-octyl phthalate	117-84-0	DOP	Olive oils		[17]
		DOP	Meat roasted		[26]
		DOP	Vegetables		[24]
		DOP	Olive oils		[22]
Di-isodecyl phthalat	26761-40-0	DiDP	Olive oils		[17]
		DiDP		Paper	[12]
		DiDP		Plastics, cardboard	[18]
		DiDP	Meat samples(roasted chicken)		[26]
Ethylene terephthalate dimers and trimers Polymer	–	PET		Plastics(PET)	[9]
Non-intentionally added substances (NIAS)					
(Z)-9-Octadecenamide	301-02-0	9-ODA		Plastics (PE)	[9]
3-[3,5-Di-tert-butyl-4-hydroxybenzyl]propionic acid	20170-32-5			Plastics (PE)	[9]
Carbonyl and vinyl species	–			Plastics (PE)	[9]
2,4-Dit-butyl-6-nitro-phenol	–	2,4di-6-P		Plastics (PE)	[9]
2,4-Dit-butyl-6-nitro-phenol and 2-cyclohexene-1-dione				Plastics (PE)	[9]
3,5-Dimethyl o-methyloxime	–	3,5 M-o-M		Plastics (PE)	[9]
Nonylphenol	25154-52-3	NP		Plastics (PET)	[9]
Octylphenol	1806-26-4	OP		Plastics (PET)	[9]
N2-dodecanoyl-l-arginine	–	LAS		Plastics (Active packaging)	[9]
1,4,7-Trioxacyclotridecane-8,13-dione	6607-34-7			Adhesives	[9]
Abietic acids	514-10-3	ABC		Adhesives	[9]
1-Hexanol-2-ethyl	104-76-7	HE		Adhesives	[9]
2-Ethylhexylacetate	103-09-3	2-Eac		Adhesives	[9]
Cyclic lactone	–	LAC		Adhesives	[9]
Nonylphenol etoxilated	–			Adhesives, Plastics (PET)	[9]
Additives					
Tinuvin 234	70321-86-17	TNV 234		Plastics (polycarbonate)	[30]
Tinuvin 326	3896-11-5	TNV 326		Plastics (polycarbonate)	[30]
Tinuvin 327	3864-99-1	TNV 327		Plastics (polycarbonate)	[30]
Tinuvin 328	25973-55-1	TNV 328		Plastics (polycarbonate)	[30]
Cyasorb UV9	131-57-7	Cys UV9		Plastics (polycarbonate)	[30]
Cyasorb UV12	131-54-4	Cys UV12		Plastics (polycarbonate)	[30]
Cyasorb UV24	131-53-3	Cys UV24		Plastics (polycarbonate)	[30]
Cyasorb UV 5411	3147-75-9	Cys UV5411		Plastics (polycarbonate)	[30]
Irgafos 168	31570-04-4	I-168		Plastics (polycarbonate)	[30]
Advastab 800	115628-90-5	Adv-800		Plastics (polycarbonate)	[30]
UVINUL 400	92092-63-2	UV-400		Plastics (polycarbonate)	[30]
Cyanox 2246	119-47-1	Cyx-2246		Plastics (polycarbonate)	[30]
Chimassorb 81	1843-05-6	Ch-81		Plastics (polycarbonate)	[30]
Uvitex OB	7128-64-05	Uv-OB		Plastics (polycarbonate)	[30]
Irganox 1076	2082-79-3	I-1076		Plastics (polycarbonate)	[30]
Irganox 1010	6683-19-8	I-1010		Plastics (polycarbonate)	[30]
Irganox 1330	1709-70-2	I-1330		Plastics (polycarbonate)	[30]
Irganox 1081	90-66-4	I-1081		Plastics (polycarbonate)	[30]
Organic contaminants					
1,2-Dimethylnaphthalene	573-98-8	12DMNa		Paper	[12]
1,4-Dimethylnaphthalene	571-58-4	14DMNa		Paper	[12]
1,6-Dimethylnaphthalene	575-43-9	16DMNa		Paper	[12]
1-Methylfluorene	1730-37-6	1mfLN		Paper	[12]

Table 1 (*Continued*)

Compound	CAS number	Abbreviation	Food product	Food contact material	Reference
1-Methylnaphthalene	90-12-0	1MNa		Paper	[12]
2,6 dimethylnaphthalene	581-42-0	26DMnA		Paper	[12]
2,7-Diisopropylnaphthalene	40458-98-8	27DiPNa		Paper	[12]
2-Methylanthracene	613-12-7	2MAnt		Paper	[12]
2-Methylnaphthalene	91-57-6	2MNa		Paper	[12]
9-Methylanthracene	779-02-2	9MAnt		Paper	[12]
Acenaphthene	83-32-9	Ace		Paper	[12]
Acenaphthylene	208-96-8	Acy		Paper	[12]
Anthracene	120-12-7	Ant		Paper	[12]
Benzo(a)anthracene	56-55-3	BaA		Paper	[12]
Dibenzo(ah)anthracene	53-70-3	BaP		Paper	[12]
Benzo(b)fluoranthene	205-99-2	bBf		Paper	[12]
Benzo(ghi)perylene	191-24-2	BghiP		Paper	[12]
Benzo(a)pyrene	50-32-8	BkF		Paper	[12]
Fluorene	86-73-7	Fln		Paper	[12]
Fluoranthene	206-44-0	Flt		Paper	[12]
Chrysene	218-01-9	Chr		Paper	[12]
Indeno(1,2,3-cd)pyrene	193-39-5	IcdP		Paper	[12]
Naphtalene	91-20-3	Na		Paper	[12]
Phenanthrene	85-01-8	Phe		Paper	[12]
Pyrene	129-00-0	Pyr		Paper	[12]
Printing inks (photoinitiators)					
2-Isopropylthioxanthone	5495-84-1	2ITX		Paper	[12]
4-Isopropylthioxanthone	83846-86-0	4ITX		Paper	[12]
4-Methylbenzophenone	134-84-9	4MBFN		Paper	[12]
Benzophenone	119-61-9	BFN		Paper	[12]
Ethyl-4-dimethylamino benzoate	10287-53-3	EDB		Paper	[12]
2-Ethylhexyl-4-dimethylamino benzoate	21245-02-3	EHDB		Paper	[12]
Mineral oil					
Mixture of saturared hydrocarbons (C16-C24)		MOSH		Recyclated Paper	[34]
Mixture of unsaturared hydrocarbons (<C24)		MOAH		Recyclated Paper	[34]

Until now, some studies have reviewed the analytical methods for phthalates [38] NIAS [9] and PFCs [39] compounds in food and FCMs. LC-MS analysis of phthalates, bisphenol A and related compounds in food-packaging materials was reviewed until 2013 by Gallart-Ayala and co-workers [4]. The present review focuses on the most recently published information for the analysis of different chemical families of food-packaging contaminants, through the identification and discussion of the most relevant methods published between 2010 and 2016. Likewise, we include for the first time the analytical methods for the determination of food-packaging contaminants using gas chromatography. Gallart-Ayala et al. reviewed [4] the analytical methods for FCMs using liquid chromatography until 2013. The present review shows the most recent advances in analytical methods using liquid chromatography (2013–2016) and as a novelty includes the most novel techniques, particularly high resolution mass spectrometry (HRMS).

In many fields such as food safety or environmental control the development of LC-HRMS platform, using both Orbitrap and TOF analyzers, has grown rapidly in the last years. However, in the FCM area it not seems so clear, or at least not so fast. We also discuss this challenge in the present work.

2. Compounds

There are hundreds of compounds that can migrate from food contact materials. In this review, we have focused on some relevant families of organic compounds (primary aromatic amines, bisphenol A, bisphenol A diglycidyl ether and related compounds, UV-ink photoinitiators, perfluorinated compounds, phthalates and non-intentionally added substances and mineral oil) where there has been increasing interest in their analysis by liquid chromatography (LC) and gas chromatography (GC) in recent years.

Several of these compounds are of particular concern because, although they are generally present in very small amounts, they are nonetheless often dangerous to human health [4].

2.1. Primary aromatic amines (PAAs)

Primary aromatic amines appear in food products through food contact materials mainly from plastics such as kitchen utensils [8] or plastic laminates [7]. These amines are formed by hydrolysis of aromatic isocyanates in polyurethane adhesives, and by degradation of azodyes used as colorants in nylon kitchen utensils and other plastic materials [40]. Brede et al. [40] identified the polyamide cooking utensils as a common source of PAAs.

Taking into account that many aromatic amines are classified as toxic compounds and/or as suspected human carcinogens [8], their migration into foodstuff from food contact material is subjected to restrictions. The European Regulation 10/2011 establishes that plastic material and articles shall not release primary aromatic amines in detectable quantity in food or food simulants. The SML are set to 0.01 mg of substances per kg of food or food simulant and it applies to the sum of primary aromatic amines (ANL, 2-4 TDA, 2-6 TDA, 4-4′MDA, 1-5DAN, m-PDA, 3-3′DCB and 4-4′DPE) released [8,35].

From these early studies onwards, numerous alerts have been issued by the European rapid alert system for food and feed (RASFF) for excessive concentrations of PAAs migrating from FCMs [41].

2.2. Bisphenols and related compounds

Bisphenol A (BPA) is used as a monomer for polycarbonate production, and it is considered to be an endocrine disrupter [4]. Recent studies highlight the potential of BPA to disrupt thyroid hormone action, to causes proliferation of human prostate cancer cells and to block testosterone synthesis [42] at very low part-per-trillion doses. Use of BPA in food contact materials is permitted in the European Union (EU) under Regulation 10/2011/EU [36]. This Regulation permits use of BPA in plastic materials and articles intended to come into contact with foodstuffs, with a SML of 0.6 mg/kg [36]. However, the European Commission adopted Directive 2011/8/EU [43] in January 2011, prohibiting its use for the manufacture of polycarbonate infant feeding bottles [4,44]. BPA has also been found in recycled paper and paperboard used for food packaging (pizza cardboard, paper bags) and in kitchen towels made from recycled paper, probably due to its use in printing inks [42]. For this reason, it is very important to establish the criteria to ensure that paper containing recycled pulp is safe enough to be used as food contact material. Further data would be needed to quantify the impact of these sources in terms of BPA exposure in the population [31]. The European Food Safety Agency conducted a risk assessment of BPA, setting a Tolerable Daily Intake of 4 μg/kg bw [4]

Other related compounds are bisphenol F (BPF), bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE). The toxicity of BPF, which has also been proven, is mainly related to its oestrogenic and antiandrogenic effects [45]. In turn, BADGE and BFDGE, have cytotoxic effects, which make them tumorigenic and mutagenic [46]. The most popular coating varnishes and lacquers used in drink and food cans are those based on vinyl organosols (novolacs), which include epoxy resins obtained from BADGE or from BFDGE in their composition. With respect to BADGEs, the EU has set SMLs of 9 mg/kg for the sum of BADGE and its hydrolyzed derivatives and 1 mg/kg for the sum of BADGE·HCl, BADGE·2HCl and BADGE·HCl·H2O [47].

2.3. Perfluorinated compounds (PFCs)

Poly- and per-fluoroalkyl substances (PFASs) are chemicals that have been found to be persistent and to bio-accumulate causing adverse human health effects [48–52]. They comprise a diverse group of compounds that have been widely used in consumer product applications such as cookware and food contact papers [53]. In fact, the chemical properties such as thermal stabilities, in addition to the hydrophobic and lipophobic nature of these compounds, have made them highly valued in consumer products [53]. Among the PFASs are the per-fluoroalkyl sulfonates (PFSAs) and the per-fluoroalkyl carboxylates (PFCAs) of which the per-fluorooctane sulfonate (PFOS) and the per-fluorooctanoic acid (PFOA) are the most important [54,55]. Recently, PFOS has been listed as a persistent organic pollutant (POP) under the Stockholm Convention on POPs in Annex B, banning their production and use [56].

Exposure to PFOS and PFOA might have occured directly due to their manufacture and use in commercial products [57]. Indirect exposure might also have occured through the release of PFOS and PFOA precursors that degrade to PFOS and PFOA, and were released into the environment during production and through treated commercial products [58–60]. Examples of such precursors include: fluorotelomer alcohols (FTOHs), perfluorooctane sulfonamides (FOSAs), and perfluorooctane sulfonamidoethanols (FOSEs). Polyfluoroalkyl phosphate esters (PAPs), mainly used in food-packing materials, are precursors of FTOHs and subsequently also of PFOA [15,61]. Recently PAPs have become a focus of attention due to their wide use in packaging materials made of paper and paperboard, including wrapping paper, milk and juice cartons, fast-food containers and microwave popcorn bags. Mono and diPAPs have been reported in food packaging materials [27,62]. EFSA has completed a risk assessment on PFOS and PFOA in the food chain and established TDIs of 150 ng/kg body weight/day and 1500 ng/kg body weight/day, respectively. [4]

2.4. Phthalates

Phthalates (PAEs) or esters of phthalic acid (1,2-benzenedicarboxylic acid) have been commonly used as plasticizers to increase flexibility, transparency, durability, and longevity of plastic materials since the 1930s [63]. Millions of tons of PAEs are produced all over the world annually, of which di-2-ethylhexyl phthalate (DEHP) is one of the most popular plasticizers and accounts for ~50% of global production, followed by dibutyl phthalate (DBP), di-iso-decyl phthalate (DIDP) and di-iso-nonyl phthalate (DINP) [4,64]. In general, the content of PAEs in plastic materials, such as polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyvinylacetates (PVA) and polyethylene (PE), varies from10% to 60% by weight [39].

Phthalates and their metabolites have been reported to cause detrimental effects to human health. For instance, researchers showed that di-n-butyl phthalate (DnBP), benzylbutyl phthalate (BBP), DEHP and DiNP can adversely affect the male reproductive system. Duty et al. [65] found an association between DNA damage in sperm and exposure to diethyl phthalate (DEP). Furthermore, Latini et al. [66] revealed that DEHP can disrupt the human endocrine system and can induce premature delivery in humans.

PAEs may easily migrate from food-packaging materials into food under certain conditions, particularly when they are in contact with fatty and oily food. However, PAEs are not labelled as ingredients on food-packaging materials [67,68]. Human exposure to PAEs occurs mainly via food ingestion [69–71]. For example, in 2011, six PAEs were detected in milk samples with plastics packaging, indicating that PAEs could migrate from plastic packaging into milk [27]. In addition to liquid milk, [72] found that milk powder was also contaminated with DEHP at the highest level of 25 μg/kg. However, PAEs in foods did not capture special attention until 2011 – when a food scandal was reported in Taiwan, revealing that DEHP was being used illegally as a clouding agent in beverages to increase profits, resulting of major public health concern [64,73,74].

In view of the potential hazards to human and animal health, some PAEs (e.g., DMP, BBP, DBP, DEP, DNOP and DEHP) have been listed as priority pollutants and their use in foods and plastic products have been restricted in the European Union (EU), USA and China [29,75,76]. Using food simulants, the European Union has established SMLs for several PAEs in food-contact materials [77–82]. For example, the SMLs (mg/kg food simulant) for BBP, DEHP and DBP are 30, 1.5 and 0.3, respectively [36]. Regarding those without SMLs, a limit of 60 mg/kg in food products is applied [83]

2.5. Non-intentionally added substances (NIAS)

Packaged food may contain non-intentionally added substances (NIAS) as a result of the interactions between different ingredients in the packaging materials, from degradation processes and mainly from the impurities present in the raw materials used for their production [84]. Most NIAS and unknown compounds are regularly detected when using high-sensitivity advanced analytical techniques, although their chemical structure is often difficult to establish. It is likely that in the majority of cases, due to the very low levels found, these substances will not be of any health concern. However, at present no guidance exists on what should be done when an unknown peak is detected.

The Regulation on Food Contact Materials (Regulation 10/2011/EU) [36] recognizes that during the manufacture and use of plastic materials and articles, NIASs can be formed. Being

relevant for risk assessment, the main NIAS of the final packaging material should be considered and included under the restrictions of the positive substance list. Any potential health risk in the final material or article arising from NIASs should be assessed by the manufacturer in accordance with internationally recognized scientific principles of risk assessment. However, NIAS identification is very difficult because of the lack of information about the real composition of the various ingredients and materials used for polymers and final packaging manufacturing. For this reason, it is almost impossible to know in detail what is included in the final packaging material.

In order to avoid the presence of NIASs in packaged food, especially when risk assessment is involved, it is very important to know their origin. Traceability of food packaging materials is obligatory and this includes a description of NIAS [9]. Degradation processes, additive degradation and impurities are important sources of NIAS. Degradation processes can take place in the polymer itself and also in the additives used for improving its physicochemical characteristics. Some additives such as antioxidants or light stabilizers added to the polymer for improving their properties can also be degraded. As a result, new potential migrants will be present in the packaging material. Some of the most common degradation products that have been studied due to toxicity are alkylphenol, nonylphenol (NP) and octylphenol (OP), which are known to be endocrine disrupters [85]. NP and OP can be generated by the oxidation of tris(nonylphenyl)phosphate (TNPP), used as an antioxidant in polymeric materials such as poly-(vinyl chloride) (PVC), polyolefins and acrylics. They can also be generated by the degradation of polyethoxylated nonylphenols (APEOs), which are common surfactants in cleaning agents used in PET (polyethylene terephthalate) bottle manufacturing and in other materials such as adhesives or polymeric dispersions [9].

Another frequent reason for finding NIAS in migration from food packaging is the presence of impurities coming from the raw materials or additives used during polymer manufacturing. Impurities from adhesive additives used in food packaging have also been found in migration. The compounds 1-hexanol-2-ethyl, 2-ethylhexylacetate and 2,4,7,9-tetramethyl-5-decyl-4,7-diol (TDMM) were found in migration from multilayer materials based on acrylic adhesives. The first two compounds were impurities from commercial 2-ethylhexylacrylate, a monomer used in the production of acrylic adhesives, and the last one was an impurity or residual surfactant monomer from ethoxylated TDMM, used as a surfactant in adhesive production [86].

2.6. Printing inks (photoinitiators)

Inks are commonly used in food packaging materials and therefore, migration of ink components to food must be studied. Printing inks (2ITX, 4ITX, 4MBFN, BFN and so on), provide information about the packaged food. When multilayer materials are used in food packaging, migration can take place, not only from the internal side of the packaging (food contact surface) but also from internal layers due to diffusion and partition processes [10,87]. In the case of inks applied on the external side of the packaging, there can be ink transference from the external side to the internal side (side in contact with food) during material production and storage in rolls, increasing the possibility of ink-component migration to food. The transference of ink components from the external printed surface of food packaging to the food contact surface is called set-off [10].

Inks, defined as a coloured fluid or paste used for writing, drawing or printing, are mainly composed by a pigment or dye, suspended or dissolved, in a solvent. The use of printing inks for food packaging is regulated by the European Printing Ink Association (EuPIA) [88]. Different groups of raw materials can be used in the manufacture of food packaging inks such as additives, colorants, (pigments, dyes), pigment additives, polymeric resins, solvents or photo-initiators [89].

Although intermediate aluminium layers are commonly used to prevent the migration of ink components into food products, the unintentional transfer of printing ink components from the outer printed surface onto the food-contact surface can occur when the printed material is rolled on spools or stacked during storage. Although ink photoinitiators are widely used, there are no specific EU controls for migration of inks and their associated coatings. A specific migration limit SML for benzophenone of 0.6 mg/kg has been established in a specific legislation for food-contact plastics [36]. Regarding those without SMLs, a limit of 60 mg/kg in food products is applied.

2.7. Additives

Additives such as antioxidants, stabilizers and plasticizers have a major influence in the processing and shelf-life of plastics and are responsible for many properties of these materials. These additives are present in small amounts in plastics (generally ranging from 0.1% and 1%), dispersed in the polymer matrix, with the aim of avoiding such effects as thermo-oxidative deterioration, which initiates scission and cross-linking of the macromolecular chains consequently leading to polymer deterioration [35]. The polymer has got an inert structure with a high molecular weight that represents a low potential risk for human health since the organism cannot absorb molecules with a molecular weight greater than 1000 Da [30]. On the contrary, as plastic additives and organic colorants have a generally low molecular weight, they may migrate from plastics into foods, representing a potential risk for human health [30].

2.8. Mineral oil

Mineral oil components found in packaging cardboard are complex mixtures of saturated hydrocarbons (MOSH) and unsaturated hydrocarbons (MOAH). MOSH are linear and branched hydrocarbons whereas MOAH are alkylsubstituted poly aromatic compounds [34].

The migration of contaminants from cardboard packaging materials into foodstuffs is a complex process. The contaminants need to be vaporized into the gas phase. From the gas phase the contaminants permeated through the functional barrier into the food. The gas phase migration process requires an evaporation of the mineral oil components with subsequent re-condensation onto packaging materials or food.

Regarding consumers safety the migration of contaminants from recycled fibres into food should be reduced down to levels below of any toxicological concern. The German Federal Ministry of Food and Agriculture (BMEL) presented a draft document for the regulation of mineral oil from recycled fibres in cardboard food packaging materials. According to this draft document, the maximum concentration of mineral oil components in cardboard packed food should be 2 mg/kg and 0.5 mg/kg for MOSH and MOAH, respectively. For MOSH the mineral oil components between n-C20 and n-C35, whereas for MOAH the compounds between C16 and C35 should be considered [34].

3. Analytical methods

3.1. Sample preparation

The analysis of FCM contaminants can be performed in food, in food simulants or in food contact materials. The analysis of contaminants in FCMs requires to perform a first stage of specific migration of substances to food simulants. There are European legislation that

regulates these migration studies. For instance, migration studies on food contact materials (plastic materials and articles not yet in contact with food) are regulated by Regulation (EU) N° 10/2011 [36]. Migration studies using food simulants are necessary in order to characterise new packaging materials and the amount of non-desirable contaminants that can migrate into food. Various food simulants, such as ethanol, acetic acid, vegetable oil and Tenax®, are listed in Regulation (EU) N° 10/2011 [36].

Recent works have described the use of these simulants in the migration of FCM contaminants. For instance, Sanchis et al. (2015) [8] studied the migration of some PAAs (primary aromatic amines) from nylon kitchen utensils following the migration test established in the European Standard EN 13130-1:2004 [14,90]. In this study, each sample was placed in a beaker which was, in turn, filled with a volume of simulant (acetic acid, 3%) enough to cover the piece of utensil used for migration. Regarding printing inks, Aznar et al. [10] carried out migration experiments with two different simulants, ethanol 95% as fat simulant and Tenax® as dry-food simulant. Two different sets of food packaging materials were studied in this work. In both studies, a polyethylene layer (PE) was in contact with food, and a printing ink was applied on the outer side in order to study the set-off effect. For ethanol experiments, pouches were filled with ethanol 95% [36]. For Tenax® migration experiments, pouches were filled with 0.64 g of Tenax®

Tables 2 and 3 show a selection of the relevant conventional and novelty analytical procedures for the three studied matrixes (food, food simulants and food contact materials) using gas and liquid chromatography, respectively, proposed in recent literature.

Some conventional techniques like Soxhlet, solid–liquid (S–L) extraction, Solid Phase Extraction (SPE), Ultrasonic extraction and liquid–liquid (L–L) extraction have been generally used for the extraction of FCM contaminants from food and food packaging (Figs. 2 and 3). Soxhlet and SPE have been used for extracting phthalates from paper materials and beverages followed by gas chromatography analysis [21,84]. For more polar compounds, determined by liquid chromatography, S–L extraction has been applied to extract BPA and BADGEs from canned food [15]. Another conventional extraction method is ultrasonic extraction. This extraction has been employed in sample preparation of phthalates from food (milk and wine) and plastic material [20,27], and in sample preparation of PFCs from paper materials [12].

Gel permeation chromatography (GPC) and gas purge microsyringe extraction (GP-MSE) clean-up steps were used for extracting phthalates from widely consumed food such as fruits, vegetables, milk, beverages, baby food and so on, after L–L and S–L extraction, respectively [18,26]. In addition, SPE has been used as clean-up method when extracting PFCs from different paper materials (baking paper, paper bags and more) after ultrasonic extraction and final determination by liquid chromatography [12].

The disadvantages of the conventional techniques are that sample preparation is usually manual and a large amount of organic solvent is required for compound extraction. In addition, a clean-up step is usually necessary when conventional extraction techniques are applied. To minimise the use of organic solvents and avoid clean-up steps, several pre-treatment methods have been described as an alternative for extraction, clean-up and concentration. Fig. 4 presents the extraction methodologies used for phthalates, PFCs, BPA, BADGEs and NIAS contaminants in food and FCMs (paper and plastics). Solid-phase microextraction (SPME) [103], focused ultrasond solid–liquid extraction (FUSLE) [104], Pressurized liquid Extraction (PLE) [105] and Quechers [106] have been currently applied to extract FCM contaminants in food and materials. SPME is a widely used technique for the analysis of volatile and semi-volatile compounds in gas chromatography [26]. SPME is commonly applied in the headspace mode (HS-SPME), in which the fibre used for extraction does not come into direct contact with the sample but with the headspace above the sample, instead. SPME can also be applied in direct-immersion mode (DI-SPME), in which the extraction fibre is placed in direct contact with the sample, with the advantage of increased extraction efficiency. DI-SPME may have an even greater efficiency when a cooling system is applied to the fibre. The extraction process consists of the analyte sorption into a fibre, an exothermic process, and cooling the fibre to accelerate the transfer of the analyte into the fibre. Recently, a solid-phase microextraction fibre cooled by liquid nitrogen (CF-SPME) was selected as a sampling technique to analyze eight plasticizers (phthalates) in spices and roasted chicken meat stored in plastic bags by gas chromatography–mass spectrometry [26]. In addition, SPME has been the selected technique to extract NIAS from plastics and cans [9]. This approach has the major advantage of no sample manipulation, therefore minimising cross contamination from glassware, solvents and samples. However, its main drawbacks are that the fibres tend to break and are relatively expensive.

FUSLE is a simple, safe and inexpensive methodology with a 100-fold higher extraction power than the traditional ultrasonic bath used as extraction technique when liquid chromatography is applied [104]. This technique has been recently used for the extraction of bisphenol-type endocrine disrupters in food-contact recycled-paper materials [31] and for perfluorinated alkyl acids in corn, popcorn and microwave popcorn packaging [32]. FUSLE factors affecting the extraction efficiency (solvent volume, extraction time and ultrasonic irradiation power) were optimized in these studies. Bisphenol-type endocrine disrupters (BPA, BPF, BADGE and BFDGE) were extracted with 20 mL methanol at the ultrasonic amplitude of 100% for 5 s. and 2 extraction cycles [31]. Selected perfluorinated alkyl acids (PFAAs) [nine perfluorocarboxylic acids (PFCAs) and perfluorooctane sulfonate (PFOS)] were efficiently extracted in a single 10-s cycle from microwave popcorn bags and from the inside popcorn before and after cooking under FUSLE conditions [32]. Better recoveries were obtained when extracting BPA, BPF, BADGE and BFDGE from recycled paper (72–97%) [31], than when extracting PFCs in corn and popcorn (65–105%) [32], when using the FUSLE extraction method.

PLE has also been used for sample treatment of BPA-related compounds and UV-ink photoinitiators in liquid chromatography [4]. This technique has recently been used for the extraction of perfluorinated compounds (PFCs) used in food packaging materials (baking paper and beverage cups) as coatings/additives for oil and moisture resistance. After a PLE extraction, a solid-phase extraction (SPE clean-up) with 1.5 g florisil, 1 g basic alumina and 1 g of sodium sulphate was applied prior to the injection into the LC–MS/MS. The PLE extraction method with a SPE clean-up obtained recoveries between 60 and 90% [13].

The Quick, easy, cheap, effective, rugged and safe (QuEChERS) method was proposed by Anastassiades et al. in 2003 [106]. The QuEChERS approach was first used to extract pesticides from foods. Nowadays, in addition to pesticides, the QuEChERS method is being increasingly applied to the extraction of other compounds. In 2014, the QuEChERS method was successfully applied to extract 23 phthalate esters (PAEs) from grape jelly, seasoning powder, egg noodles and grapefruit sauce [97]. In 2016, QuEChERS was applied after ultrasonic extraction using a mixture of acetonitrile and water for the determination of 68 potential contaminants: specifically phthalates, polycyclic aromatic hydrocarbons, photoinitiators, bisphenols and polyfluorinated compounds in paper FCMs [12]. Quechers has been used in FCMs methodologies as a extraction and clean-up technique obtaining good recoveries (75–115%) as an extraction technique in food samples for phthalates [33,97] and as a clean-up technique for PFCs in paper materials (70–120%) [12] by liquid chromatography. However, when extract-

Table 2
Analysis of food packaging contaminants in food, food simulants and packaging materials by gas chromatography and mass spectrometry detection.

Compound	Food simulant	Food product	Food contact material	Extraction	Clean-up	Technique	GC conditions	Ionisation source	Recovery	LOD	LOQ	Refs.
Phthalates: DEA,DiBA, DBA, DMP,DEP, DiBP, DBP, BBP,DiHP, DEHP, DOP, DiNP,DiDP, DEHS	–	Olive oil	–	Liquid–liquid extraction with Acetonitrile	–	HRGC–MS	Supelco SPB-5MS (5%polydiphenylsiloxane,95% polydimethylsiloxane) (0.25 μm × 30 mm × 0.25 mm)	EI mode	93–101%	0.003–1.200 mg/kg	0.001–4 mg/kg	[17]
Phthalates, organic contaminants and photoinitiators: Na, 2MNa,1MNa, 26DMNa,16DMNa,14DMNa,12DMNa,Acy,DMP,Ace, DEP, Fln,27DiPNa, BFN,1MFln,EDB, 4MBFN, DiBP, Phe,Ant, DBP, 2MAnt,9MAnt, Ant,DNPP, Flt, Pyr, EHDB, DEHP,DiNP, 4ITX, 2ITX,DiDP, BaA,DNOP,Chr,BbF,BkF,BaP, IcdP, BghiP.	–	–	Paper	Liquid–liquid extraction with Quechers	–	GC–MS/MS	Rxi–PAH column (0.10 μm × 30 mm × 0.25 mm)	EI mode	52–135%		0.001–0.22 mg/kg	[12]
Phthalates: DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP, DNOP	–	Fruits, vegetables, milk, cereals, meat, fish, fat, oils, snacks, condiments, sauces, beverages, miscella-neous and baby food	–	Liquid–liquid extraction	gel per-meation chro-matog-raphy (GPC)	GC–MS	DB–XLB column (0.25 μm × 60 mm × 0.25 mm)	EI/CI MSD mode	89–101%		0.01–145 μg/kg	[18]
Phthalates: DMP, DEP, DiBP, DnBP, BBP, DEHP, DCHP, DNOP	60 min with 40 mL n-hexane in an ultrasonic bath	–	Plastics, paperboard		–	GC–MS	DB–XLB column (0.25 μm × 60 mm × 0.25 mm)	EI/CI MSD mode	82–99%		0.1–1.5 μg/kg	[18]
Phthalates: DEP,BBP, DEHP, DBP DOP, DiBP, DCHP	–	Meat samples (roasted chicken)	–	Solid-phase microex-traction	fibre cooled by liquid nitro-gen (CF-SPME)	GC–MS	HP-5MS Agilent column (0.25 μm × 30 mm × 0.25 mm)	EI mode		0.01–0.18 μg/kg	0.07–0.26 μg/kg	[26]

Table 2 (*Continued*)

Compound	Food simulant	Food product	Food contact material	Extraction	Clean-up	Technique	GC conditions	Ionisation source	Recovery	LOD	LOQ	Refs.
Phthalates ans esters: DEP, DEHP,DBP, DNOP, DMP,BBP	–	78 samples of widely consumed food	–	Solid-phase extraction	gas purge microsyringe extraction (GP-MSE)	GC–MS	DB5 fused-silica capillary column (0.25 μm × 30 mm × 0.25 mm)	EI mode	90–100%	0.14–0.38 ng/g for solid 0.0021–0.0096 ng/mL for liquid		[26]
Phthalates: DMP,DEP,DBP,BBP,DNOP	–	Bottled milk	–	Ultrasound-assisted dispersive liquid–liquid microextraction	–	GC–MS	KB-1 (0.25 μm × 30 mm × 0.25 mm)	–	93–106%	0.64–0.79 ng/g		[27]
Phthalates: DMP,DEP,DIBP,DBP,DAP, DHP,BBP,BBEP,DEHP,DOP	–	Olive oil	–	Headspace solid-phase microextraction	–	GC–MS	ZB-5MS (0.25 μm × 30 mm × 0.25 mm)	–			0.02–0.05 mg/kg	[22]
Phthalates: DMP,DBP,DEP,DEHP	–	Fatty food	–	Liquid–liquid extraction	–	GC–MS	DB-5 MS (0.25 μm × 30 mm × 0.25 mm)	–		0.4 μg/g	1.2 μg/g	[25]
Phthalates: DMP,DBP,DEP,BBP,DIBP, DEHP	–	Wine	–	Ultrasound-vortex-assisted dispersive liquid–liquid microextraction	–	GC–MS	SE-54 (0.25 μm × 30 mm × 0.25 mm)	–	85–100%	0.0022 μg/L	0.075 μg/L	[20]
Phthalates: DMP,DEP,DBP,DAP,DNOP	–	Soybean milk	–	Molecularly imprinted solid-phase extraction (MISPE)	–	GC–MS	DB-5MS (0.25 μm × 30 mm × 0.25 mm)	–	76–108%	0.013–0.022 μg/mL		[23]
Phthalates: DMP, DEP, DBP, BBP, DIBP, DEHP	–	Hydroalcoholic food beverages	–	Solid phase extraction (SPE) with Amberlite XAD-2 adsorbent.	–	GC–MS	SE-54 (0.24 μm × 30 mm × 0.25 mm)	–	94–103%	1.21–2.51 pg/μL	2.42–5.03 pg/μL	[21]
Phthalates: DBP,DEHP	–	Chicken soup	–	Magnetic micro-solid phase extraction	–	GC–MS	DB-5(0.25 μm × 30 mm × 0.25 mm)	–	70–118%	26.3–36.4 μg/mL		[28]
Phthalates: DMP,DEP,DBP,BBP,DEHP,DOP	–	Vegetables	–	Stir bar sorptive extraction	–	GC–MS	DB-17 MS (0.25 μm × 30 mm × 0.25 mm)	–	83–118%	15.8–106 pg/g		[24]
Phthalates: MP,DEP,DBP,DIBP,DMEP,DNOP	–	Fatty foods	–	Dispersive solid phase extraction	–	GC–MS	DB-5 MS (0.25 μm × 30 mm × 0.25 mm)	–	71–115%	0.4–0.8 μg/mL		[19]
Phthalates: DMP, DEP, DBP,DIBP,DMEP,BBP, DHXP, DCHP, DNOP	–	Various food	–	Liquid–liquid extraction	gel permeation chromatography (GPC)	GC–MS	DB-5 MS (0.25 μm × 30 mm × 0.25 mm)	–		1.5 mg/kg (fatty food) 0.05 mg/kg other food		[29]
Ethyl carbamate	–	Alcoholic beverages and soy	–	Solid-phase extraction	–	GC–MS	DB Innowax capillary (1.25 μm × 30 mm × 0.25 mm)	–	96–107%		5 μg/kg	[91]

Additives: I-168, I-1076, TNV 326, Ch-81	24 h at 60 °C, total inmersion with hexane	–	Polypropylene films	–	–	HPLC-GC	12 m × 0.25 mm column coated with a 0.13 μm film of PS-255, a cross linked dimethyl polysiloxane	Flame ionisation detection (FID)		0.1–1 mg/kg		[11]
BPA, DEHP	–	–	Paperboard	BPA-extraction in ethanol, DEHP in acetone–hexane 4:1, Soxhlet extraction	–	GC–MS	SLB-5ms type (5%polysilarylene-95% polydimethylsiloxane) (0.25 μm × 30 mm × 0.25 mm)	–	101–108%	0.015–0.017 mg/L	0.05–0.064 mg/L	[84]
BPA, DEHP	migration with simulant Tenax	Salt,sugar	–	–	–	GC–MS	SLB-5ms type (5%polysilarylene-95% polydimethylsiloxane) (0.25 μm × 30 mm × 0.25 mm)	–	70–120%	0.015–0.017 mg/L	0.05–0.064 mg/L	[84]
BPA, DEHP	migration with simulant Tenax	–	Recycled paper and paperboard	–	–	GC–MS	Supelco SLB-5ms type (5% polysilarylene–95% polydimethylsiloxane; (0.25 μm × 30 mm × 0.25 mm)	–	70–120%		0.06–25 mg/L for BPA, 0.5–60 mg/Lfor DEHP	[92]
Ethyl acetate, Methyl methacrylate, Toluene, Hexanal, Paraldehyde, P-xylene, Butyl acrylate, Styrene, P-cymene, 2-octanone, 1-hexanol, 2-ethylhexylacetate, Nonanal, Cyclohexanol, Acetic acid, 2-ethyl-1-hexanol, Camphor, Propanoic acid, Benzaldehyde, 1-octanol, butyric acid, Methylben-zoate, naphtalene, allylbenzoate.	Migration with Tenax in the oven 40 °C for 10 days	–	Multilayer materials for packaging dry food	–	–	GC–MS	BP-20 (0.25 μm × 30 mm × 0.25 mm)	–		0.01–25.7 μg/g		[93]

Table 2 (*Continued*)

Compound	Food simulant	Food product	Food contact material	Extraction	Clean-up	Technique	GC conditions	Ionisation source	Recovery	LOD	LOQ	Refs.
Volatile compounds: hexanal, octanal, 2-heptenal, 1-hydroxy-2-propanone, nonanal, 2-octenal, furfural, decanal, 2-nonenal, 2-furanmethanol, 2-decenal, 2,4 decanienal isomers, hexanoic acid	Migration with Tenax in the oven 40 °C for 10 days	Powdered milK	–	–	–	GC–MS	DB WAX (0.20 μm × 50 mm × 0.40 μm)	–				[94]
Melamine		Powdered milK		Liquid–liquid extraccction	–	GC–MS	HB-5MS (0.25 μm × 30 mm × 0.25 mm)	EI mode	95–101%		0.025 mg/kg	[95]
Perfluorinated compounds: FTOHs, FOSAs, and FOSEs	Two migrations,first acetic 3%, second methanol	–	–	–	SPE (solid phase extraction)	GC–MS	DB-WAX column (0.25 μm × 30 mm × 0.25 mm)	Positive chemical ionisation (PCI)		3.9–30 pg		[96]
133 Volatile compounds (esters, acids, saturated alcohols, unsaturated aliphatic alcohols, ketones,aldehydes, furans,ethers,lactones,sulphur containing compounds)	–	Camel milk	–	Solid phase microextraction, solvent assisted flavour evaporation, and simultaneus destillation extraction.	–	GC–MS	DB-WAX column (0.25 μm × 30 mm × 0.25 mm)	–				[97]
Mineral oil: MOSH, MOAH	–		paperboard	Liquid–liquid extraction with etanol–hexane 1:1 during three days	–	HPLC–GC-FID	HPLC column (25 cm × 2 mm, silica gel) and a 10 m × 0.25 mm separation column coated with a 0.13 m film of PS-255 in the GC	–		0.1 mgc/kg	–	[98]
Mineral oil: MOSH, MOAH		food	paperboard	Food: extraction with hexane, 8 hours. Paperboard: extarction with etanol–hexane 1:1 2 hours	–	HPLC–GC-FID	HPLC column (25 cm × 2 mm, silica gel); GC: Separation columns were coated with a dimethyl polysiloxane stationary phase (KLZH: 10 m × 0.25 mm, 0.13 m PS-255; BfR: DB1-HT, 15 m × 0.32 mm, 0.10 m)	–	72.9% MOSH, 27.1% MOAH	0.5 mg/kg		[99]
NIAS	Migration with Tenax		Acrylic adhesives	HS-SPME extraction and liquid extraction	–	APGC–MS(Q-TOF)	HP-5MS (30 m × 0.25 m × 250 mm)	Atmospheric-pressure gas chromatography (APGC)		2.2–4.3 μg/kg		[100]

Table 3
Analysis of food packaging contaminants in food, food simulants and packaging materials by liquid chromatography and mass spectrometry detection.

Compound	Food simulant	Food product	Food contact material	Extraction	Clean-up	LC conditions	Technique	Ionisation source	Recovery	LOD	LOQ	Refs.
Amines: ANL,2,4 TDA, 2,6 TDA, m-PDA, 1,5 DAN, 3,3′ DMB, 4,4′DPE,4,4′ MDA	Migration with acetic acid 3%, 2 hours at 100 °C	–	Nylon kitchen utensils	–	–	C18 Phenyl hexil column (2.1 μm x 100 × 2.1 mm), Methanol 2/water 98	LC–HRMS	ESI(+)	70–120%		2.5 μg/kg	[8]
Amines: ANL, 2,4 TDA, BNZ, o-ANS,4,4′ DPE, o-TOL, 4,4′ MDA,o- DANS, o-TOLI, p-ANL, p-CRS, 4,4′ MBM,4,4′ TDA, 2-NAPH, 4-Cl-TOL, 5-N-O-TOL, 2,4,5 MTA, 4-ABF,4,4′ M(2Cl),3,3′ DCB,p-ABZ,o-AaT	Migration:0.6 dm of plastic laminate was cut and then inmersed in 100 mL de acetic 3% the solution incubated 70 °C for 2 h.	–	Plastic laminate	–	–	C18 (2.6 μm × 100 × 2.1 mm) water 4.7 mM perfluropropanoic acid(PPFPA)/4.7 mM PFPA en methanol	HPLC–HRMS	ESI(+)		0.06–5.27 μg/kg	0.09–5.45 μg/kg	[7]
BPA, BADGEs and related compounds: BPA, BPF,BADGE,BFDGE	–	–	Recycled paper	Focused ultrasonic solid–liquid extraction (FUSLE)	–	C18 (1.7 μm × 50 × 2.1 mm) ACN:water/0.5 mM sodium acetate 8.5 mM acid acetic	UPLC–Q-TOF-MS	ESI(+)	72–97%	16–47 ng/mL		[31]
BPA, BPB, BPF, BPE, BFDGE, BADGE, BADGE-H_2O, BADGE-$2H_2O$, BADGE-H–O–HCl, BADGE-HCl, BADGE-2HCl, BFDGE-2HCl	–	Canned food	–	Solid–liquid microex-traction	–	C18 (5 μm × 250 × 4.5 mm)ACN/ water in isocratic conditions	Liquid chromatography fluorescence detection		80–110%		0.9–3.5 μg/kg	[15]
BPA,BADGE	–	–	Polycarbonate	Chloroform/ Methanol	–	C18 Acclaim PepMapRSLC (2 μm × 150 × 0.3 mm)1 mM ammonium formate in 10:90 methanol:water (v/v)/1 mM ammonium formate inmethanol	UHPLC	ESI(+)(–)	95–98%			[30]

Table 3 (*Continued*)

Compound	Food simulant	Food product	Food contact material	Extraction	Clean-up	LC conditions	Technique	Ionisation source	Recovery	LOD	LOQ	Refs.
Perfluorinated compounds: Perfluorooctanesulfonic acid tetraethylammonium salt PFOS, perfluorobutanoic acid PFBA, perfluoropentanoic acid PFPeA, perfluorohexanoic PFHxA acid, perfluroheptanoic acid PFHpA, perfluorooctanoic acid PFOA, perfluorononanoic acid PFNA, perfluorodecanoic acid PFDA, perfluorounde-canoic acid PFUnA, perfluorododecanoic acid PFDoA	–	–	Microwave popcorn bags	Focused ultasound solid–liquid extraction (FUSLE)	–	C18 (1.7 μm × 50 × 2.1 mm) 0.8% formic–acetonitrile)0.8% formic	UHPLC–QTOFMs/Ms analysis	ESI(−)	80–106%	0.2–0.5 ng/g	0.4–1.6 ng/g	[32]
Perfluorinated com-pounds:PFOS, PFBA,PFPeA, c PFHxA, PFHpA,PFOA, PFNA, PFDA, PFUnA, PFDoA	–	Corn and popcorn	–	Focused ultasound solid–liquid extraction (FUSLE)	–	C18 (1.7 μm × 50 × 2.1 mm) 0.8% formic–acetonitrile)0.8% formic)	UHPLC–QTOFMs/Ms analysis	ESI(−)	65–105%	0.2–0.7 ng/g	0.2–0.6 ng/g	[32]
Perfluorinated com-pounds:PFBA, PFOA, PFPeA, PFHxA, PFHpA, PFNA, PFDA, PFUnDA, PFDOA, PFBS, PFHxS, PFOS, PFTrDA, PFTeDA, PFHxDA, PFODA, PFDS	–		Aluminium foil wrap-pers,baking paper materials or beverage cups	Pressurized liquid extraction (PLE) with methanol and	Clean-up with Florisil-Basic Alu-mina column	Hypersil GOLD C8 (3 μm,150 mm, 2.1 mm) 5 mM ammonium acetate–MeOH	LC–MS/MS analysis		60–90%	from 0.20 to 0.94 ng/g		[13]
PFCs, PFCA,PFAAS,PFAPA,PAP,FOSA,PFCs, and bisphenols	–		Paper,Cardboard, Paper filters for coffee	Ultrasonic extraction by mixture of acetonitrile and water	Clean-up with quech-ers	EC-C18 column (2.7 μm, 150 mm, 3 mm)	HPLC–MS/MS		70–120%		0.0027–0.13 mg/kg	[12]

PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFBuS, PFHxS, PFHpS, PFOS	–	Chicken eggs		Solid–liquid extraction with methanol solvent.	–	C18 column (5 μm, 50 mm, 2.1 mm)/ammonium formate in water/methanol.	LC–MS/MS analysis		90–120%	0.15 ng/g	0.5 ng/g	[16]
Phthalates:DMP, DMEP, DEEP, DEP, DAP, DIPrP, DPrP, DPhP, DIBP, BBP, DIPP, DPP, DCHP, DMPP, DHXP, DHP, DEHP, DNOP, DINP, DNP, DIPP.	–	Food samples including milk-based products, distilled liquor, wine, beverage, grain, meat, oil, biscuit (cookie), and canned food.		Liquid samples extracted by acetonitrile. Solid samples by QuEChERS of glass-based SPE methods	–	Poroshell 120 EC-C18 column (100 × 4.6 mm, 2.7 μm)	LC–MS/MS analysis		75.5–115.2%	0.8–15 μg/kg	10–100 μg/kg	[101]
Phthalates:DMP,DEP,DiBP, DnBP, BBP, DEHP, DOP, DiNP, DiDP		wine		Liquid–liquid extraction	–	Synergi Hydro-RP HPLCcolumn with a (2 mm, 4 μm, 80A) 10 mM ammoniumacetate/methanol	HPLC–MS/MS		95–105%		1.6–26.6 μg/L	[102]
Phthalates:DMeP, DMP, DMEP, DEEP,DEP, DAP, DIPrP, DPrP, DPhP, DBP, DIBP, DBEP,DBeP,BBP, DBuP, DIPP, DPP,DCHP, BMPP, DHXP, DHP,DEeP, DINP, DNP,DIDP, (DNOP) and bis(2-DEHP.	–	Milk and milk products		Liquid–liquid extraction with quechers	–		UHPLC/ESI Q-Orbitrap)	ESI(+)(–)	90,7–104.6%	0.32–2.6 μg/kg		[33]
Ink and photoinitiatos: 3,90.217.1080, 4,62.285,1315, 5,35.251,1260, 5,44.453.1770, 5,50.473.1448, 5,71.337,1627, 6,50.273.2067, 6,50.259.1911, 6,82.389.1118, 6,97.403.2334, 7,05.287,2230, 7,19.297.2412, 7,22.341.2655, 7,23.385,2928, 7,36.315.2549, 7,39.315.2549, 7,62.343.2855, 7,80.371.3174	Migration With simulants ethanol 95% and Tenax		Multilayer material	-	-	C18 (1.7 μm × 100 × 2.1 mm) water 0.1% formic/methanol 0.1% formic)	UPLC–QTOF-Ms analysis	ESCI				[10]

Table 3 (*Continued*)

Compound	Food simulant	Food product	Food contact material	Extraction	Clean-up	LC conditions	Technique	Ionisation source	Recovery	LOD	LOQ	Refs.
Non-intentionally added substances (NIAs): Formaldehyde Polymer (PET), Acetaldehyde Polymer (PET), Ethylene terephthalate dimers and trimers Polymer (PET), 2,4-di-tertbutyl-phenol (2,4-DBTP) Polymer (pp), 2,6-di-tertbutyl-p-benzoquinone (2,6-DTBQ) polymer (PP), 3,5-ditertbutyl-4-hydroxyphenylpropionic acid polymer (PP), 2,6-ditertbutyl-4-methoxyphenol polymer (PP), 3,5-ditertbutyl-4-hydroxybenzoic acid polymer (PP), triph-enylphosphate polymer (PP), tri-o-tolylphosphate polymer (PP), diphenylphos-phate polymer (PP), dimethyl-benzaldehyde polymer (PP), 4-hydroxy-1H-indole-3-carboxilic acid polymer (PP), 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione polymer (PP), methyl-3-(3,5-ditertbutyl-4-hydroxyphenyl)propionate Polymer (PP),												

3-[3,5-di-tert-butyl-4-hydroxybenzyl]propionic acid polymer (PP), carbonyl and vinyl species polymer (PE), (Z)-9-octadecenamide polymer (PE), 2,4-dit-butyl-6-nitro-phenol polymer (PE), 2,4-dit-butyl-6-nitro-phenol and 2-cyclohexene-1-dione, 3,5-dimethyl, o-methyloxime polymer (PE), nonylphenol (NP) Polymer (PET), Octylphenol (OP) polymer (PET), primary aromatic amines (PAAS),N2-dodecanoyl-l-arginine (LAS), C10H16O2 active packaging, 1,4,7-trioxacyclotridecane-8,13-dione adhesives, BADGE derivates, ESBO clorohydrins PVC, Abietic acid derivates adhesives, 1-hexanol-2-ethyl adhesives, 2-ethylhexylacetate adhesives, cyclic lactone Adhesives, nonylphenol etoxilated	Polymers: PET,PP,PE,Printed materials, active packaging, adhesives, cans, PVC	Headspace, Solid Phase Microextraction, Liquid-Liquid extraction, Liquid-liquid Microextraction	volatiles GC–MS(QTOF), non volatiles LC–MS(QTOF)	ESI	[9]

Table 3 (*Continued*)

Compound	Food simulant	Food product	Food contact material	Extraction	Clean-up	LC conditions	Technique	Ionisation source	Recovery	LOD	LOQ	Refs.
Additives: BHT,BHA, TNV 234, TNV 326, TNV 327, TNV328, Cys UV 9, Cys UV12, Cys UV 24, Cys UV 5411, I-168, Adv-800, UV-400, Cyx-2246, Ch-81,Uv-OB, I-1076, I-1010, I-1330, I-1081	-		polycarbonate	Chloroform/Methanol		C18 (2,0 μm × 150 × 0.3 mm) ACN:water/0.5 mM sodium acetate 8.5 mM acid acetic	UHPLC–HRMS	ESI(+)(–)	95–98%			[30]

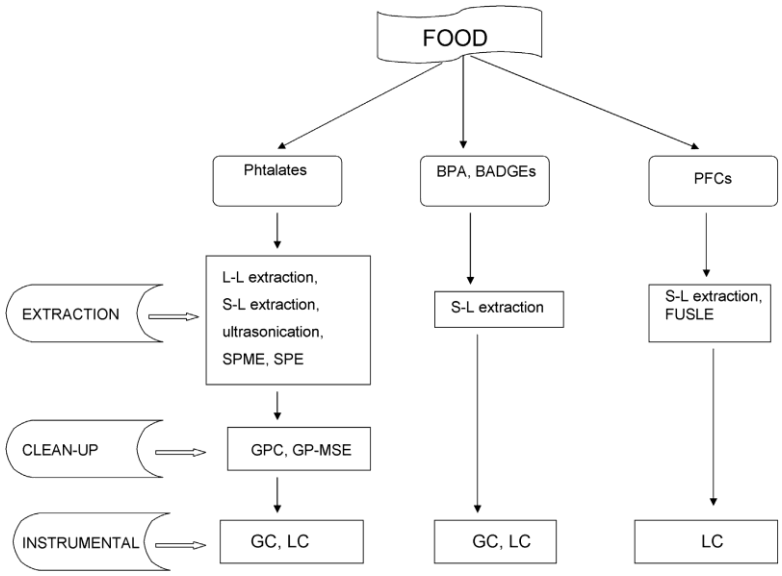


Fig. 2. Analytical strategies (extraction, clean-up and instrumental techniques) for food samples.

ing phthalates from paper materials using gas chromatography, poor recoveries were described (52–135%) [12].

In summary, in the field of FCMs. migration studies using food simulants are regulated for some FCMs. However, it will be interesting that these studies will be regulated in a wider scope of FCMs. Regarding to extraction methodologies for FCM contaminants in food and FCMs (paper and plastics) there are not a clear trend because some conventional techniques are still applied in this field. As a novelty, QuEChERS has been used in FCM methodologies as a extraction and clean-up technique. In general, few clean-up steps are applied after the extraction of FCM contaminants. Trends towards sample preparation automation and minimisation of organic solvent use are observed (PLE, SPME, FUSLE). Overall, good recoveries are generally obtained for all families of compounds using the selected methodologies.

3.2. Instrumental techniques

The selection of the instrumental technique depends on the physicochemical properties of the target substances and their concentration. For the analysis of FCM organic contaminants, both GC and LC are commonly used coupled to mass spectrometry detectors (MS and MS/MS). GC is the technique best suited for the apolar and volatile compounds (e.g., phthalates). However, the second technique, LC, is selected for more polar compounds with lower volatility or lower thermal stability, including PFCs, PAAS, NIAS and photoinitiators.

Tables 2 and 3 summarise the analytical techniques by GC and LC, respectively, and their main characteristics used in recently published studies for the analysis of the food-packaging contaminants addressed in this review, in food simulants, food and different food contact materials.

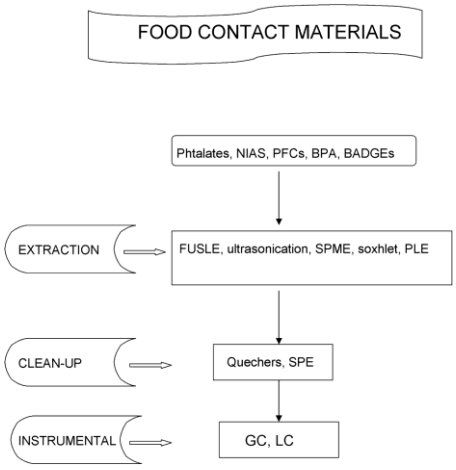


Fig. 3. Analytical strategies (extraction, clean-up and instrumental techniques) for food contact materials.

3.2.1. Gas chromatography (GC)

Phthalates and BPA are the families of organic FCM compounds mainly analyzed by GC (Fig. 5, Table 2). Recently published methods have a narrow scope, of usually between 1 and 25 of these compounds. This is in clear contrast with the present tendency observed in other fields such as water that presents a prevalence

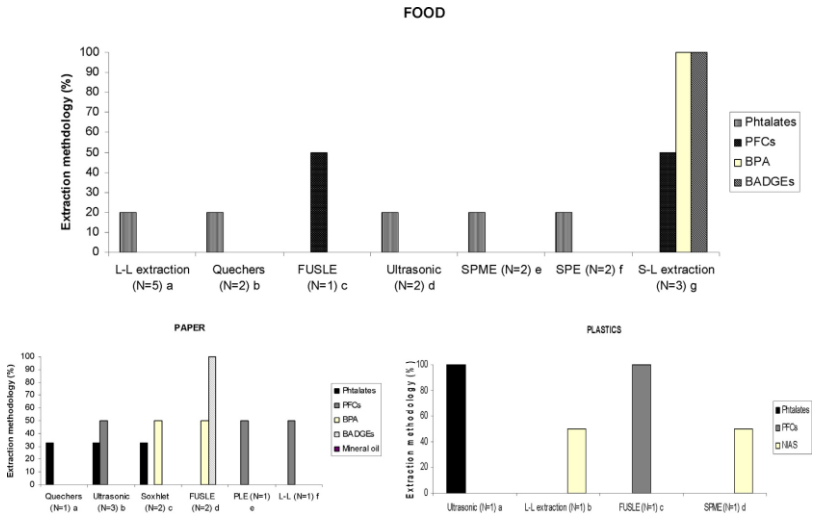


Fig. 4. Publications on methodologies to extract contaminants from food and materials (paper and plastic). (*N* = number of papers published for each extraction technique; Letters = references). *Food*: a: [17,18,25,27,29]; b: [12,33]; c: [32]; d: [20,27]; e: [22,26]; f: [21,101]; g: [15,16,32]. *Paper*: a: [12]; b: [12,18,97]; c: [84,97]; d: [31,32]; e: [13], f: [98]. *Plastic*: a: [18]; b: [9]; c: [32]; d: [9].

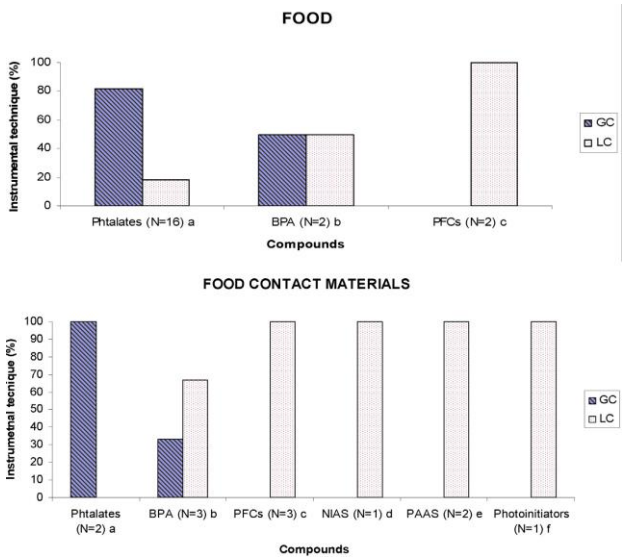


Fig. 5. Publications on Instrumental techniques used in food and food contact materials for some families of compounds. (*N* = number of papers published for each compound; Letters = references). *Food*: a: [17,18,19,20,21,22,23,24,25,26,27,28,29,33,101,102]; b: [15,84]; c: [16,32]. *Food contact materials*: a: [12,18]; b: [31,84,92]; c: [12,13,32]; d: [9]; e: [7,8]; f: [10].

of multiresidue/multiclass methods for more than 150 substances on average [107].

Phthalates have been detected in oil [17,22], milk [27], meat [18], beverages [19], wine [20], vegetables [19] and fat [25]) and in FCMs (paper [12] and plastics [18]) using GC–MS In general, the polarity of analytes is the most important parameter for the selection of an analytical column. Due to the relatively low polarity of PAEs, a non-polar column (5% phenyl-95%dimethylpolysiloxane)

and a mid-polar column (50% phenyl-50% dimethylpolysiloxane) are frequently used [108] Table 2 shows typical GC methods reported for the analysis of PAEs in foods and FCMs. Electron Impact (EI) is the ionisation technique most commonly applied. Since the contents of PAEs in food samples are generally at ultratrace levels, highly-sensitive detectors are indispensable for positive identification and quantification. Consequently, GC–MS, has been the main approach to determine PAEs due to its high sensitivity and reliability [17–28,29]. In addition, the enhanced selectivity (using SIM mode) is another advantage of the MS detector, which reduces the requirement for chromatographic separation and increases the sensitivity of PAE detection to some extent. In paper packing materials, a mass spectrometry in tandem mode was developed for the analysis of PAEs (GC–MS/MS) [12]. Good sensitivity and recoveries were obtained for PAEs in food samples ranging from 70% in chicken soup [28] to 118% in chicken soup and vegetables [24]. In materials, migration of PAEs from cardboard, tetrabrick and plastics presented good recoveries ranging from 82 to 99% and low detection limits using GC–EI-MS [18]. However, in paper materials the recoveries obtained were poorer (52–135%) but limits of detection were also low employing the GC–MS/MS technique [12].

The high efficiency and low cost of plastics materials containing phthalates stimulate their wide use in various practical applications, leading to their ubiquity in laboratories. In general, excessively high background signals account for poor LODs. Accordingly, sample contamination by these compounds cannot be ignored in the analysis. According to the literature, seven major measures were advised to avoid the contamination with phthalates: (1) all plastics consumables should be avoided; (2) organic solvents used should be purified with aluminium oxide; (3) all glassware should be cleaned up with blank tested organic solvents (e.g., acetone, hexane) and dried prior to use; (4) all reagents, including laboratory water, should be tested to establish the blank value; (5) all clean laboratory consumables should be kept in a desiccator containing aluminium oxide to avoid recontamination; (6) the chromatographic system, especially the inlet and the caps of sampler vials, should be checked initially and regularly by injecting the blank for indication of the contamination levels; and (7) use of personal-hygiene products containing PAEs should be avoided [38].

BPA has been analyzed in salt and sugar and in paperboard by gas chromatography [84]. In all these matrices a 5% polysilarylene-95% polydimethylsiloxane column and EI ionisation source were employed. Simple mass spectrometry quadrupole (MS) was using providing low detection limits, and a LOQ between 0.05 and 0.064 mg/L in food and packing material was obtained [84].

Apart from mass spectrometry detection (applied to phthalates and BPA analysis) other detectors were employed to analyze NIAS in polypropylene films. GC-FID provided good LOD (0.1–1 mg/kg) in these plastic matrices. Mineral oil in paperboard was also detected using on line HPLC-GC-FID with LOD of 0.1 mg/kg [98].

In addition, atmospheric pressure gas chromatography coupled to a quadrupole-time of flight mass spectrometry (APGC-MS/Q-TOF) has demonstrated to be a powerful tool for indentification of NIAS in acrylic adhesives used in food packaging materials. The results were compared to those obtained in a target analysis by conventional GC–MS-Q (quadrupole), and three new compounds were identified and their structure were elucidated working with the spectra obtained by APGC-MS/Q-TOF in a non-target analysis [100].

In summary, GC analytical methodologies mainly developed in FCMs field are coupled to low resolution mass spectrometers (LRMS) working in SIM [22,26,27]. More recently, GC in tandem (MS/MS) mode [12] have been also employed. No trend was observed in the studied period (2010–2016) towards the use of HRMS detection, where only one study analyzed NIAS in acrylic adhesives used in food packaging materials with Q-TOF detector [100].

3.3. Liquid chromatography (LC)

Gallart-Ayala et al. reviewed [4] the analytical methods for FCMs using liquid chromatography until 2013. They concluded that MS/MS (QqQ) continues to be the method of choice in the analysis of food packaging contaminants. The present review shows the most recent advances in analytical methods using liquid chromatography (see Table 3). Although MS/MS continues to be the method of choice, the use of HRMS is one of the best ways to prevent false positives or even false negatives, and we present some relevant examples concerning the analysis of FCM organic contaminants.

LC–MS has been the selected technique in the analysis of PFCs, PAAS and photoinitiators in plastic packaging [7,10,12,13,32], plastic utensils [8] (see Fig. 5). In addition, LC has been used for the analysis of PFCs in food such as corn and popcorn [32] and chicken eggs [16], as well as of BPA and BADGEs in canned food [15] and of phthalates in milk-based products [33], beverages [101,102], grain, meat, oil, biscuit and canned food [101], wine [102] (see Table 3). Similarly to GC, recently published methods in LC, have a narrow scope, of usually between 1 and 25 of compounds.

Generally, column sizes are in the range of 100–250 mm, with a particle size from 2 to 5 μm (see Table 3) [7,10,31]. This is a typical LC set-up for the determination of a low number of compounds (e.g. <50). As previously mentioned by Gallart-Ayala et al. [4], ultra-high performance liquid chromatography (UHPLC) is the most convenient approach to achieve reliable, fast LC separations in the analysis of food-packaging contaminants, because of lower particle size (<2.1 μm), and better resolution is provided. According to Gallart-Ayala et al. [4], RP separations continue to be the chromatographic mode of choice for the analysis of many of these compounds.

Acetonitrile and methanol continues to be the organic components of the mobile phases currently used in LC. Besides, solvent modifiers, mainly formic acid and PFPA as a proton donors, are added for enhancing ionisation efficiencies or improving peak separation or peak shape of target compounds.

Regarding ionisation of food packaging contaminants in LC, electrospray ionisation (ESI) is the most commonly used technique. The positive-ionisation mode is usually employed to analyze PAAS, BADGEs and BFDGEs, UV-ink photoinitiators, and phthalate diesters, while the negative ionisation mode gives the best sensitivity for the detection of phthalate-monoester metabolites, BPA, other bisphenols (e.g., BPE, BPB, BPF and BPS) and PFCs (Table 3). In general, negative ESI and positive ESI are dominated by the deprotonated molecule, $[M-H]^-$, or the protonated molecule, $[M+H]^+$, respectively, and no further fragmentation is usually observed. However, in-source fragmentation can occasionally be observed, e.g., with some UV-ink photoinitiators (2-Hydroxy-2-methylpropiophenone (HMPP), 1-Hydroxycyclohexyl phenyl ketone (HCPK), 2,2-dimethoxy-2-phenylacetophenone (DMPA), 4,4.-Bis(diethylamino)-benzophenone (DEAB)) [109] In some cases, the formation of adduct ions with components of the mobile phase was also observed. BADGEs and BFDGEs showed a high tendency to form adducts such as $[M+Na]^+$, $[M+K]^+$, $[M+NH_4]^+$ and $[M+ACN]^+$ ions. However, some of these cluster ions {e.g., $[M+Na]^+$} are very stable and no further fragmentation in MS/MS was obtained, but efficient fragmentation occurred for ammonium adducts with a stable signal under MS/MS [110,111]. In these cases, to enable the formation of ammonium adducts and ensure signal reproducibility, formic acid/ammonium formate buffer is generally used as an additive in the mobile phase in positive ESI for the analysis of these compounds.

Although MS/MS continues to be the method of choice, the use of HRMS method using TOF or Orbitrap mass analysers has been recently introduced for the analysis of inks [109], PFCs[32] additives [30] and phatalates [33] in FCMs. This technique has allowed the identification of NIAS such as unknown molecules possibly deriving from polycarbonate degradation [30] or phthalates possibly deriving from nylon kitchen utensils in a non-target analysis [8]. In target analysis, PFCs were analyzed by UHPLC–QTOF-MS/MS in corn, popcorn and popcorn bags [32]. Better recoveries were obtained in microwave popcorn bags (80–106%) than in food matrices (corn and popcorn) (65–105%), although a better LOQ was validated in food (0.2–0.6 ng/g) [32]. Mattarozzi et al. [7] developed a target methodology for the analysis of 22 PAAS from plastic laminates by LC–HRMS, with the LOQ ranging between 0.099–5.45 μg/kg [7]. LC–HRMS has also been used for determination of PAAS from nylon kitchen utensils [8], achieving an LOQ of 2.5 μg/kg.

4. Conclusions and future trends

In this work, we have reviewed the recent analytical strategies for the most relevant food-packaging-contaminant families (PAAS, BPA, BADGEs and related compounds, UV-ink photoinitiators, PFCs, phthalates and NIAS), in FCMs, food and food simulants. For some contaminants (NIAS, PAAS and photoinitiators) studies have only focused on FCMs. More studies on food samples will be necessary in the near future.

Armonization will be necessary for sample preparation and analytical methods for the analysis of FCMs in food, food simulants and food contact materials. PLE, SPME, FUSLE and Quechers are sample preparation methods recently used in these type of analysis due to the integration of the extraction and clean-up procedures, automation of sample preparation and minimisation of organic solvent use. FCMs contaminants in food simulants are regulated, but more legislation will be necessary in food and FCMs. However, there is a lack of regulation about the most suitable extraction and clean-up technique to be applied in these matrixes. SML are regulated for many substances and groups of them, but more specific or individual migration level will be interesting to be established in the near future.

The GC–MS technique is mainly used for the analysis of phthalates, BPA and NIAS in food and food packaging materials. On the other hand, for the more polar substances including PFCs, PAAS and photoinitiators, LC-MS/MS has been the selected technique. Standarization of the analytical methods for FCMs in food simulants, food and food contact materials will be also useful in future.

A clear trend towards the application of HRMS in LC for target, post-target and non-target analysis has been observed in the last three years (since 2013). However, this tendency was not observed in the case of GC. Recently, the LC–HRMS method using TOF or Orbitrap mass analysers has been introduced for the analysis of some FCMs contaminants such as PFCs, PAAs, additives and phatalates in FCMs. Nowadays, the analytical challenges in this field should be the development of FCMs contaminants methodologies in food, food simulants and food contact materials applying the HRMS technique.

References

[1] R. Coles, D. McDowell, M.J. Kirwan, Food Packaging Technology, second ed., 2003, Oxford.
[2] P. Muniandy, A.B. Shori, A.S. Baba, Influence of green, white and black tea addition on the antioxidant activity of probiotic yogurt during refrigerated storage, Food Packag. Shelf Life 8 (2016) 1–8.
[3] G. Koutsimanis, K. Getter, B. Behe, J. Harte, E. Almenar, Influences of packaging attributes on consumer purchase decisions for fresh produce, Appetite 59 (2012) 270–280.
[4] H. Gallart-Ayala, O. Nunez, P. Lucci, Recent advances in LC-MS análisis of food-packaging contaminants, Trends Anal. Chem. 42 (2013) 99–124.
[5] European commission, Commission Regulation (EU) No. 1935/2004 of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC, Off. J. Eur. Union L338 (2004) 1.
[6] Commission Regulation (EC), No 2023/2006 of December 2006 on good manufacturing practice for materials and articles intended to come into contact with food, Off. J. Eur. Union L384 (2006) 1.
[7] M. Monica, L. Francesca, S. Michele, C. Maria, Liquid chromatography – full scan-high resolution mass spectrometry-based method towards the comprehensive analysis of migration of primary aromatic amines from food packaging, J. Chromatogr. A 1320 (2013) 96–102.
[8] Y. Sanchis, C. Coscollà, M. Roca, V. Yusà, Target analysis of primary aromatic amines combined with a comprehensive screening of migrating substances in kitchen utensils by liquid chromatography-high resolution mass spectrometry, Talanta 138 (2015) 290–297.
[9] C. Nerin, P. Alfaro, M. Aznar, C. Domeño, The challenge of identifying non-intentionally added substances from food packaging materials: a review, Anal. Chim. Acta 775 (2013) 14–24.
[10] M. Aznar, C. Domeño, C. Nerín, O. Bosetti, Set-off of non volatile compounds from printing inks in food packaging materials and the role of lacquers to avoid migration, Dyes Pigments 114 (2015) 85–92.
[11] R. Castillo, M. Biedermann, A.M. Riquet, K. Grob, Comprehensive on-line HPLC–GC for screening potential migrants from polypropylene into food: the effect of pulsed Light decontamination as an example, Polym. Degrad. Stab. 98 (2013) 1679–1687.
[12] L. Vavrous, A. Vapenka, J. Sosnovcov, K. Kejlov, K. Vrbík, D. Jírov, Method for analysis of 68 organic contaminants in food contact paper using gas and liquid chromatography coupled with tandem mass spectrometry, Food Control 60 (2016) 221–229.
[13] E. Zafeiraki, D. Costopoulou, I. Vassiliadou, E. Bakeas, L. Leondiadis, Determination of perfluorinated compounds (PFCs) in various foodstuff packaging materials used in the Greek market, Chemosphere 94 (2014) 169–176.
[14] C. Simoneau, (EURL) at col. EN 24815 EN 2011, JRC Scientific and technical reports, 2011.
[15] A. Alabi, N. Caballero-Casero, S. Rubio, Quick and simple sample treatment for multiresidue analysis of bisphenols, bisphenol diglycidyl ethers and their derivatives in canned food prior to liquid chromatography and fluorescence detection, J. Chromatogr. A 1336 (2014) 23–33.
[16] E. Zafeiraki, D. Costopoulou, V. Irene, L. Leondiadis, E. Dassenakis, R. Hoogenboom, S. Leeuwen, Perfluoroalkylated substances (PFASs) in home and commercially produced chicken eggs from the Netherlands and Greece, Chemosphere 144 (2016) 2106–2112.
[17] G. Dugo, V. Fotia, V. Turco, R. Maisano, A.G. Potortì, A. Salvo, G. Di Bella, Phthalate, adipate and sebacate residues by HRGC-MS in olive oils from Sicily and Molise (Italy), Food Control 22 (2011) 982–988.
[18] T. Fierens, K. Servaes, M. Van Holderbek, L. Geerts, S. De Henauw, I. Sioen, G. Vanermen, Analysis of phthalates in food products and packaging materials sold on the Belgian market, Food Chem. Toxicol. 50 (2012) 2575–2583.
[19] L.P. Zhu, T. Zhu, Y.P. Ma, Y.F. Ni, Y. Wang, Q.C. Yan, Rapid determination of 15 kinds of phthalate esters in vegetable juice sby hollow fiber-liquid phase microextraction coupled with gas chromatography–mass spectrometry, Anal. Chem. 41 (2013) 1019–1024.
[20] G. Cinelli, P. Avino, I. Notardonato, A. Centola, M.V. Russo, Rapid analysis of six phthalate esters in wine by ultrasound-vortex-assisted dispersive liquid–liquid micro-extraction coupled with gas chromatography-flame ionization detector or gas chromatography-ion trap mass spectrometry, Anal. Chim. Acta 769 (2013) 72–78.
[21] G. Cinelli, P. Avino, I. Notardonato, A. Centola, M.V. Russo, Study of XAD-2 adsorbent for the enrichment of trace levels of phthalate esters in hydroalcoholic food beverages and análisis by gas chromatography coupled with flame ionization and ion-trap mass spectrometry dettectors, Food Chem. 146 (2014) 181–187.
[22] J.J. Rios, A. Morales, G. Marquez-Ruiz, Headspace solid-phase microextraction of oil matrices heated at high temperature and phthalate esters determination by gas chromatography–multistage mass spectrometry, Talanta 80 (2010) 2076–2082.
[23] J. He, R. Lv, K. Lu, Selective solid-phase extraction of dibutyl phthalate from soybean milk using molecular imprinted polymers, Anal. Chim. Acta 661 (2010) 215–221.
[24] J.L. Cacho, N. Campillo, P. Vinas, M. Hernandez-Cordoba, Determination of alkylphenols and phtalate esters in vegetables and migration studies from their packages by means of stir bar sorptive extraction coupled to gas chromatography–mass spectrometry, J. Chromatogr. A 1241 (2012) 21–27.
[25] I. Ostrovsky, R. Cabala, R. Kubinec, R. Gorova, J. Blasco, J. Kubincova, L. Rimnacova, W. Lorenz, Determination of phthalate sum in fatty food by gas chromatography, Food Chem. 124 (2011) 392–395.
[26] M.A. Moreira, L. Coelho, Z.L. Cardeal, Analysis of plasticiser migration to meat roasted in plastic bags by SPME–GC/MS, Food Chem. 178 (2015) 195–200.
[27] H. Yan, X. Cheng, B. Liu, Simultaneous determination of six phthalate esters in bottled milks using ultrasound-assisted dispersive liquid–liquid microextraction coupled with gas chromatography, J. Chromatogr. B 879 (2011) 2507–2512.
[28] F. Makkliang, P. Kanatharana, P. Thavarungkul, C. Thammakhet, Development of magnetic micro-solid phase extraction for analysis of phthalate esters in packaged food, Food Chem. 166 (2015) 275–282.

[29] Standard of the People's Republic of China, Determination of phthalate esters in foods, GB/T 21911-2008, 2008.
[30] C. Bignardi, A. Cavazza, C. Corradini, P. Salvadeo, Targeted and untargeted data-dependent experiments for characterization of polycarbonate food-contact plastics by ultra high performance chromatography coupled to quadrupole orbitrap tandem mass spectrometry, J. Chromatogr. A 1372 (2014) 133–144.
[31] D. Perez-Palacios, M.A. Fernandez-Recio, C. Moreta, M.T. Tena, Determination of bisphenol-type endocrine disrupting compounds in food-contact recycled-paper materials by focused ultrasonic solid–liquid extraction and ultra performance liquid chromatography–high resolution mass spectrometry, Talanta 99 (2012) 167–174.
[32] C. Moreta, M.T. Tena, Determination of perfluorinated alkyl acids in corn, popcorn and popcorn bags before and after cooking by focused ultrasound solid–liquid extraction, liquid chromatography and quadrupole-time of flight mass spectrometry, J. Chromatogr. A 1355 (2014) 211–218.
[33] W. Jia, X. Chu, Y. Ling, J. Huang, J. Chang, Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole orbitrap high-resolution mass spectrometry, J. Chromatogr. A 1362 (2014) 110–118.
[34] H. Diehl, F. Welle, How to determine functional barrier performance towards mineral oil contaminants from recycled cardboard, Food Packag. Shelf Life 5 (2015) 41–49.
[35] A. Sanches Silva, R. Sendón García, I. Cooperb, R. Franz, P. Paseiro Losada, Compilation of analytical methods and guidelines for the determination of selected model migrants from plastic packaging, Trends Food Sci. Technol. 17 (2006) 535–546.
[36] European commission, Commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Off. J. Eur. Union L12 (2011) 1.
[37] K. Marsh, B. Bugusu, Food packaging-roles, materials, and environmental issues, J. Food Sci. 72 (2007) 39–55.
[38] J. Yang, Y. Li, Y. Wang, J. Ruan, J. Zhang, C. Sun, Recent advances in analysis of phthalate esters in foods, TrAC Trends Anal. Chem. 72 (2015) 10–26.
[39] Y. Pico, M. Farre, M. Llorca, D. Barcelo, Perfluorinated compounds in food: a global perspective, Crit. Rev. Food Sci. Nutr. 7 (2011) 605–625.
[40] C. Brede, I. Skjervak, H. Herikstad, Determination of primary aromatic amines in water food simulant using solid-phase analytical derivatization followed by gas chromatography coupled with mass spectrometry, J. Chromatogr. A 983 (2003) 35–42.
[41] Rapid Alert System for Food and Feed (EU), AnnualReport2008, 2008, Available online at: http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2008_en.pdf.
[42] L. Gao, J. Zou, H. Liu, Y. Wang, X. Chen, Determination of bisphenol A in thermal printing papers treated by alkaline aqueous solution using the combination of single-drop microextraction and HPLC, J. Sep. Sci. 36 (2013) 1298–1303.
[43] European Commission, Commission Directive 2011/8/EU of 28January 2011 amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles, Off. J. Eur. Commun. L26 (2011) 11–14.
[44] O. Núñez, H. Gallart-Ayala, C.P.B. Martins, P. Lucci, New trends in fast liquid chromatography for food and environmental analysis, J. Chromatogr. A 1228 (2012) 298–323.
[45] K. Satoh, K. Ohyama, N. Aoki, M. Iida, F. Nagai, Study on anti-androgenic effects of bisphenol a diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and their derivatives using cells stably transfected with human androgen receptor, AR-EcoScreen, Food Chem. Toxicol. 42 (2004) 983–993.
[46] J. Míguez et al, A LC–MS/MS method for the determination of BADGE-related and BFDGE-related compounds in canned fish food samples based on the formation of $[M+NH_4]^+$ aducts, Food Chem. 135 (2012) 1310–1315.
[47] European Commission, Commission Regulation (EC) No 1895/2005 of 18 November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food, Off. J. Eur. Commun. L302 (2005) 28.
[48] U.N. Joensen, R. Bossi, H. Leffers, A.A. Jensen, N.E. Skakkebæk, N. Jørgensen, Do perfluoroalkyl compounds impair human semen quality? Environ. Health Perspect. 117 (2009) 923–927.
[49] J.W. Nelson, E.E. Hatch, T.F. Webster, Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population, Environ. Health Perspect. 118 (2010) 197–202.
[50] L. Tao, J. Ma, T. Kunisue, E.L. Libelo, S. Tanabe, K. Kannan, Perfluorinated compounds in human breast milk from several Asian countries, and in infant formula and dairy milk from the United States, Environ. Sci. Technol. 42 (2008) 8597–8602.
[51] R. Dallaire, E. Dewailly, D. Pereg, S. Dery, P. Ayotte, Thyroid function and plasma concentrations of poly halogenated compounds in inuit adults, Environ. Health Perspect. 117 (2009) 1380–1386.
[52] A.K. Rosenmai, F.K. Nielsen, M. Pederse, N. Hadrup, X. Trier, J.H. Christensen, A.M. Vinggaard, Fluorochemicals used in food packaging inhibit male sex hormone synthesis, Toxicol. Appl. Pharmacol. 266 (2013) 132–142.
[53] R.C. Buck, J. Franklin, U. Berger, J.M. Conder, I.T. Cousins, P. de Voogt, A.A. Jensen, K. Kannan, S.A. Mabury, Van Leeuwen, perfluoroalkyl and polyfluoroalkylsubstances in the environment: terminology, classification, and origins, Integr. Environ. Assess. Manage. 7 (2011) 513–541.
[54] K. Kannan, S. Corsolini, J. Falandysz, G. Fillmann, K.S. Kumar, B.G. Loganathan, M.A. Mohd, J. Olivero, N. Van Wouwe, J.H. Yang, K.M. Aldous, Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries, Environ. Sci. Technol. 38 (2004) 4489–4495.
[55] European Parliament, Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, evaluation, authorisation and restriction of chemicals (REACH), Official J. Eur. Union (2006, December), L396/1eL396/849.
[56] UNEP, Governments Unite to Step-up Reduction on Global DDT Reliance and Add Nine New Chemicals under International Treaty, 2009, http://chm.pops.int/Convention/Pressrelease/COP4Geneva8May2009/tabid/542/language/en-US/Default.aspx (accessed 09.03.15).
[57] E. Sinclair, S.K. Kim, H.B. Akinleye, K. Kannan, Quantitation of gas-phase perfluoroalkyl surfactants and fluorotelomer alcohols released from non-stick cookware and microwave popcorn bags, Environ. Sci. Technol. 41 (2007) 1180–1185.
[58] J.W. Martin, D.A. Ellis, S.A. Mabury, M.D. Hurley, T.J. Wallington, Atmospheric chemistry of perfluoroalkanesulfonamides: kinetic and product studies of the OH radical and Cl atom initiated oxidation of N-ethyl perfluorobutanesulfonamide, Environ. Sci. Technol. 40 (2006) 864–872.
[59] K. Prevedouros, I.T. Cousins, R.C. Buck, S.H. Korzeniowski, Sources, fate and transport of perfluorocarboxylates, Environ. Sci. Technol. 40 (2006) 32–44.
[60] J.C. D'Eon, M.D. Hurley, T.J. Wallington, S.A. Mabury, Atmospheric chemistry of N-methyl perfluorobutane sulfonamidoethanol, $C_4F_9SO_2N(CH_3)CH_2CH_2OH$: kinetics and mechanism of reaction with OH, Environ. Sci. Technol. 40 (2006) 1862–1868.
[61] H. Lee, J.C. D'Eon, S.A. Mabury, Biodegradation of polyfluoroalkyl phosphates as a source of perfluorinated acids to the environment, Environ. Sci. Technol. 44 (2010) 3305–3310.
[62] W.A. Gebbink, S. Ullah, O. Sandblom, U. Berger, Polyfluoroalkyl phosphate esters and perfluoroalkyl carboxylic acids in target food samples and packaging method development and screening, Environ. Sci. Pollut. Res. 20 (2013) 7949–7958.
[63] H. Farahani, M.R. Ganjali, R. Dinarvand, P. Norouzi, Screening method for phthalate esters in water using liquid–phase microextraction based on the solidification of a floating organic microdrop combined with gas chromatography–mass spectrometry, Talanta 76 (2008) 718–723.
[64] A. Espachs-Barroso, R.C. Soliva-Fortuny, O. Martin-Belloso, A natural clouding agent from orange peels obtained using polygalacturonase and cellulose, Food Chem. 92 (2005) 55–61.
[65] S. Duty, A.M. Calafat, M.J. Silva, L. Ryan, R. Hauser, The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay, Hum. Reprod. 20 (2003) 604–610.
[66] G. Latini, A. Verrotti, C. De Felice, Di-2-ethylhexyl phthalate and endocrine disruption: a review, Curr. Drug Targets Immune Endocr. Metabol. Disord. 4 (2004) 37–40.
[67] R.E. Dodson, M. Nishioka, L.J. Standley, L.J. Perovich, J.G. Brody, R.A. Rudel, Endocrine disruptors and asthma-associated chemicals in consumer products, Environ. Health Perspect. 120 (2012) 935–943.
[68] Y.Y. Fan, S.H. Liu, Q.L. Xie, Rapid determination of phthalate esters in alcoholic bever-ages by conventional ionic liquid dispersive liquid–liquid microextraction coupled with high performance liquid chromatography, Talanta 119 (2014) 291–298.
[69] X.L. Cao, Phthalate esters in foods: sources, occurrence, and analytical methods, Compr. Rev. Food Sci. Food Saf. 9 (2010) 21–43.
[70] H. Fromme, L. Gruber, M. Schlummer, G. Wolz, S. Boehmer, J. Angerer, Intake of phthalates and di(2 ethylhexyl)adipate: results of the integrated exposure assessment survey based on duplicate diet samples and biomonitoring data, Environ. Int. 33 (2007) 1012–1020.
[71] E. Fasano, F. Bono-Blay, T. Cirillo, P. Montuori, S. Lacorte, Migration of phthalates, alkylphenols, bisphenol A and di(2-ethylhexyl)adipate from food packaging, Food Control 27 (2012) 132–138.
[72] A. Khedr, Optimized extraction method for LC–MS determination of bisphenol A, mela-mine and di(2-ethylhexyl) phthalate in selected soft drinks, syringes, and milk powder, J. Chromatogr. B 930 (2013) 98–103.
[73] The Codex Committee on Food Additives and Contaminants, General standard for food additives. CODEX STAN 192–1995, 1995.
[74] National Standards of the People's Republic of China, Standards for use of food additives. GB 2760-2011, 2011.
[75] E.J. Hoekstra, J.H. Pertersen, J. Bustos, Guidance document on fat reduction factor, functional barrier concept, phthalates and primary aromatic amines, European Commission JRC Scientific and Technical Reports, JRC 68007 EUR 25112 EN, 2011.
[76] M. Llompart, C. Garcia-Jares, P. Landin, in: L.M.L. Nollet (Ed.), Phthalate esters, Marcel Dekker Inc., New York, 2005, pp. 1103–1153.
[77] European Food Safety Authority (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to di-butylphthalate (DBP) for use in food contact materials, Question N° EFSA-Q-2003-192, EFSA J. 242 (2005) 1–17.
[78] European Food Safety Authority (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to butylbenzylphthalate (BBP) for use in food contact materials, Question N° EFSA-Q-2003-190, EFSA J. 241 (2005) 1–14.
[79] European Food Safety Authority (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to

di-isononylphthalate (DINP) for use in food contact materials, Question N° EFSA-Q-2003-194, EFSA J. 244 (2005) 1–18.
[80] European Food Safety Authority (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di-isodecylphthalate (DIDP) for use in food contact materials, EFSA J. 245 (2005) 1–14.
[81] European Food Safety Authority (EFSA), Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials, Question N° EFSA-Q-2003-191, EFSA J. 243 (2005) 1–20.
[82] European Union, Commission Directive 2007/19/EC. Amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs, Off. J. Eur. Commun. L.91 (2007) 17–36.
[83] European Food Safety Authority, Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) on the reclassification of some phthalates for consistency with the new SCF guidelines for food contact materials, 2004 (Expressed on 26.05.04).
[84] M. Aznar, A. Rodriguez-Lafuente, P. Alfaro, C. Nerin, UPLC-Q-TOF-MS analysis of non-volatile migrants from new active packaging materials, Anal. Bioanal. Chem. 404 (2012) 1945–1957.
[85] C. Bach, X. Dauchy, M.-C. Chagnon, S. Etienne, Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephtalate (PET) bottles: a source of controversy reviewed, Water Res. 46 (2012) 571.
[86] E. Canellas, C. Nerin, R. Moore, P. Silcock, New UPLC coupled to mass spectrometry approaches for screening of non-volatile compounds as potential migrants from adhesives used in food packaging materials, Anal. Chim. Acta 666 (2010) 62.
[87] F.I. Andrade, M.I.F. Guedes, I.G.P. Vieira, F.N.P. Mendes, P.A.S. Rodrigues, C.S.C. Maia, Determination of synthetic food dyes in commercial soft drinks by TLC and ionpair HPLC, Food Chem. 157 (2014) 193–198.
[88] European Printing Ink Association, EUPIA guideline on printing inks applied to the non-food contact surface of food packaging materials and articles, 2011.
[89] European Printing Ink Association, EUPIA, Inventory list comprising packaging ink raw materials applied to the non-food contact surface of food packaging, 2013.
[90] Technical Guidelines on Testing the Migration of Primary Aromatic Amines from Polyamide Kitchenware and of Formaldehyde from Melamine Kitchenware (EUR24815EN), 2011.
[91] Z. Huang, X.D. Pan, P.G. Wu, Q. Chen, J.-L. Han, X.-H. Shen, Validation (in-house and collaboratory) of the quantification method for ethyl carbamate in alcoholic beverages and soy sauce by GC–MS, Food Chem. 141 (2013) 4161–4165.
[92] N.A. Suciu, F. Tiberto, S. Vasileiadis, L. Lamastra, M. Trevisan, Recycled paper-paperboard for food contact materials: contaminants suspected and migration into foods and food stimulant, Food Chem. 141 (2013) 4146–4415.
[93] P. Vera, E. Canellas, C. Nerín, Migration for odorous compounds from adhesives used in market samples of food packaging materials by chromatography olfatometry and mass spectrometry (GC–O–MS), Food chem. 145 (2014) 237–244.
[94] I. Francesca, P. Patrizia, C. Luca, M. Federico, R. Analiza, Analysis of volatile compounds in powdered milk for infant nutrition by direct desorption (CIS4–TDU) and GC–MS, Talanta 141 (2015) 195–199.
[95] P. Lutter, M.C. Savoy-Perroud, E. Campos-Gimenez, L. Meyer, T. Goldmann, M.C. Bertholet, P. Mottier, A. Desmarchelier, F. Monard, C. Perrin, F. Robert, T. Delatour, Screening and confirmatory methods for the determination of melamine in cow's milk and milk-based powdered infant formula: validation and proficiency-tests of ELISA, HPLC–UV, GC–MS and LC–MS/MS, Food Control 22 (2011) 903–913.
[96] T. Shoeib, Y. Hassan, C. Rauert, T. Harner, Poly-and perfluoroalkyl substances (PFASs) in indoor dust and food packaging materials in Egypt: trends in developed and developing countries, Chemosphere 144 (2015) 1573–1581.
[97] L. Ning, Z. Fu-ping, C. Hai-tao, L. Si-yuan, G. Chen, S. Bao-guo, Identification of volatile components in Chinese Sinkiang fermented camel milk using SAFE, SDE, and HS-SPME-GC/MS, Food Chem. 129 (2011) 1242–1252.
[98] M. Biedermann, K. Grob, Assurance of safety of recycled paperboard for food packaging through comprehensive analysis of potential migrants is unrealistic, J. Chromatogr. A 1293 (2013) 107–119.
[99] K. Fiselier, F. Grundböck, K. Schön, O. Kappenstein, K. Pfaff, C. Hutzler, A. Luch, K. Grob, Development of a manual method for the determination of mineral oil in foods and paperboard, J. Chromatogr. A 1271 (2013) 192–200.
[100] E. Canellas, P. Vera, C. Domeño, A.P. Alfaro, C. Nerín, Atmospheric pressure gas chromatography coupled to quadrupole-time of flight mass spectrometry as a powerful tool for identification of non intentionally added substances in acrylic adhesives used in food packaging materials, J. Chromatogr. A 1235 (2012) 141–148.
[101] D.M. Xu, X.J. Deng, E.H. Fang, X.H. Zheng, Y. Zhou, L.Y. Lin, et al., Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry, J. Chromatogr. A 1324 (2014) 49–56.
[102] Y. Havasaka, Analysis of phthalates in wine using liquid chromatography tandem mass spectrometry combined with a hold-back column: Chromatographic strategy to avoid the influence of pre-existing phthalate contamination in a liquid chromatography system, J. Chromatogr. A 1372 (2014) 120–127.
[103] J. Pawliszyn, Theory of solid-phase microextraction, J. Chromatogr. Sci. 38 (2000) 271–278.
[104] FUSE and ultrasound-assisted extraction for food and environmental samples, TrAC Trends Anal. Chem. 43 (2013, February) 84–99.
[105] P. Vazquez, Y. Picó, Pressurized liquid extraction of organic contaminants in environmental and food samples, TrAC Trends Anal. Chem. 71 (2015) 55–64.
[106] M. Anastassiades, S.J. Lehotay, D. Stajnbaher, F.J. Schenck, Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce, J. AOAC Int. 86 (2003) 412–431.
[107] V. Yusa, M. Millet, C. Coscolla, M. Roca, Analytical methods for human biomonitoring of pesticides: a review, Anal. Chim. Acta 891 (2015) 15–31.
[108] M. Mezcua, M.A. Martinez-Uroz, M.M. Gomez-Ramos, M.J. Gomez, J.M. Navas, A.R. Fernandez-Alba, Analysis of synthetic endocrine-disrupting chemicals in food: a review, Talanta 100 (2012) 90–106.
[109] H. Gallart-Ayala, O. Nuñez, E. Moyano, M.T. Galceran, Analysis of UV ink photoinitiators in packaged food by fast liquid chromatography at sub-ambient temperature coupled to tandem mass spectrometry, J. Chromatogr. A 1218 (2011) 459–466.
[110] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Fast liquid chromatography–tandem mass spectrometry for the analysis of bisphenol A-diglycil ether, bisphenol F-diglycidyl ether and their derivates in canned food and bevereges, J. Chromatogr. A 1218 (2011) 1603–1610.
[111] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Multiple-stage mass spectrometry analysis of bisphenol A diglycidyl ether, bisphenol F diglycidyl ether and their derivatives, Rapid Commun. Mass Spectrom. 24 (2010) 3469–3477.

2. Objetivos

La presencia de contaminantes orgánicos en materiales en contacto con alimentos (envases, embalajes, utensilios…) puede suponer un riesgo de migración de éstos a los alimentos, y ser una fuente de exposición humana a los mismos.

La presente Tesis Doctoral tiene como objetivo principal el desarrollo de nuevas metodologías analíticas basadas en la cromatografía líquida acoplada a la espectrometría de masas para la determinación de contaminantes orgánicos procedentes de los materiales destinados a entrar en contacto con alimentos.

Este objetivo principal se compone a su vez de los siguientes objetivos específicos:

1. Realizar una revisión crítica del estado actual de las metodologías analíticas para la determinación de contaminantes orgánicos presentes en los materiales en contacto con los alimentos.
2. Desarrollo de una metodología analítica que combine el análisis cuantitativo de aminas aromáticas con un cribado amplio de contaminantes sospechosos, en utensilios alimentarios de poliamida.
3. Desarrollo de una metodología analítica para la determinación cuantitativa de aminas aromáticas y fotoiniciadores, y el análisis retrospectivo de contaminantes en envases alimentarios.
4. Diseño de una estrategia analítica para la determinación cuantitativa de bisfenoles (BPA, BPF y BPS) y parabenos (MP, EP, PP, BP) en orinas.
5. Evaluación de la exposición interna a bisfenoles y parabenos en una población de madres lactantes

3. Material y métodos

3.1. Materiales, reactivos, patrones y equipos

3.1.1. Materiales

- Vasos de precipitados de vidrio 500 y 250 ml
- Papel aluminio
- Gomas
- Pipetas Pasteur
- Tubos de vidrio de topacio de 15ml con tapones de rosca
- Homogeneizadores cerámicos para tubos de 50 ml de Agilent
- Tubos falcon de 50 ml
- Tubos de polipropileno de 15ml con tapones de rosca
- Tubos eppendorf de 0,5 ml para microcentrífuga con filtros de 0,22 mm.
- Probeta graduada de 500 ml
- Matraces aforados clase A de 5,10, 25, 50 y 100 ml

3.1.2. Reactivos

- Metanol para análisis de residuos (PAR) y LC-MS
- Acetonitrilo para análisis de residuos (PAR) y LC-MS
- Acetato de etilo para análisis de residuos (PAR)
- Agua grado HPLC
- Ácido acético suprapur
- Ácido fórmico suprapur
- Acetato amónico para HPLC (97%).
- Formiato amónico solución Ultra (100 ml, 10M en agua).
- Enzima hidrolítica β-glucuronidasa aril sulfatasa
- Cloruro sódico
- Hidróxido sódico

- QuEChERS salt Kit EN (4 g $MgSO_4$, 1 gr de NaCl, 1 gr de citrato sódico, 0.5 gr de citrato sódico sesquihidrato) de Agilent Technologies.

3.1.3. Patrones

Los patrones utilizados en los métodos analíticos de la presente Tesis Doctoral corresponden a distintas familias de contaminantes (aminas, derivados epoxídicos, fotoiniciadores, PFAs, PFRs, bisfenoles y parabenos).

Los compuestos utilizados en cada grupo de familias son los siguientes:

Aminas: 2, 6 toluendiamine 98% (2-6 TDA), 2, 4 toluendiamine 98% (2-4 TDA), anilina 98% (ANL), 1,5 Naphtalenediamina 99% (1-5 DAN),1, 3 phenylenediamine (m-PDA) 99%, 4, 4 ' diaminonaphenylether (4, 4 ' DPE) 98%, y 3, 3 ' dimetilbenzidina (3,4 ' DMB) 98%, fueron suministradas por Sigma Aldrich (Barcelona, España).

Derivados epoxídicos: Bisfenol A diclicidil éter (BADGE) > 95%, Bisfenol F diclicidil éter (BFDGE) > 95%, Bisfenol A (2,3-dihidroxipropil) glicidil éter (BADGE· H_2O) >95%, Bisfenol A bis(2,3-dihidroxipropil) glicidil éter (BADGE· $2H_2O$) >95%, Bisfenol A (3-cloro-2-hidroxipropil) glicidil éter (BADGE·HCl) > 95%, Bisfenol A bis (3-cloro-2-hidroxipropil) éter (BADGE·2HCl) >95%, Bisfenol A (3-cloro-2-hidroxipropil) (2,3-dihidrocropil) éter (BADGE· HCl· H_2O) >95%, fueron suministrados por Fluka (Buchs, Suiza).

Fotoiniciadores de alta pureza: 1-hidroxiciclohexyl fenil cetona 99% (HCPK), 2, 2-dimetoxi-2-Fenilacetofenona 98% (DMPA), 2,4-Diethyl9H-tioxanthen-9-1 98% (DETX), 2-ethylhexyl 4-dimetilamino-benzoato 98% (EHDAB), 2- Hidroxi-2-metilpropiophenona 99% (HMPP), isopropil Thioxanthone 99% (ITX), 4,4 '-bis-dimetilamino-benzofenona 98% (DEAB), 4-benzoyibifenilo 98% (PBZ) y benzophenona 99% (BP), suministrados por Sigma Aldrich (Barcelona, España) y Dr. Ehrenstorfer (Augsburg, Alemania).

Retardantes de llama (PFR): 2-etilhexil difenil fosfato (EHDPP), tris (2-coloroisopropil) fosfato (TCPP) y trifenil fosfato (TPhP) de alta pureza (> 95%) y suministrados por el Dr. Ehrenstorfer (Ausburg, Alemania) y Sigma-Aldrich (Barcelona, España).

Los compuestos perfluorados (PFAS), ácido perfluorooctanoico (PFOA) y sulfonato de perfluorooctano (PFOS) de purezas > 95% se obtuvieron de Wellington Laboratories (Guelp, ON, Canadá).

Fenoles: Bisfenol A 97% (BPA), Bisfenol F 98% (BPF) y Bisfenol S >95% (BPS), suministrados por el Dr. Ehrenstorfer (Ausburg, Alemania).

Parabenos: Metilparabeno > 95% (MP), Etilparabeno > 95% (EP), Propilparabeno > 95% (PRP) y butilparabeno >95% (BP) suministrados por Sigma-Aldrich (Barcelona, España). Sal sódica de bisfenol S monosulfato, bis (4-hidroxifenil) sulfona O-β-D-glucurónido Sal sódica, bisfenol F mono-β-D-glucurónida, bisfenol F monosulfato sódico sal, bisfenol A monosulfato sódico sal, bisfenol A β-D -Glucuronide fue suministrado por Toronto research Chemicals (Canadá).

3.1.4. Equipos

- Estufa para migración Selecta calibrada con rango de temperaturas hasta 110ªC
- Placa calefactora (Mettler-Toledo)
- Evaporador TurvoVap LV (Zymark Corporation, Framingham, MA)
- Balanza analítica de precisión 0,0001 g (Mettler-Toledo)
- Microcentrífuga con refrigeración 13000 rpm (Eppendorf)
- Centrífuga de 11000 rpm (Jouan)
- Centrífuga de 4000 rpm (Beckman)
- Micropipetas de volumen variable entre 10-100 µl, 20-200 µl, 100-1000 µl y 1-10 ml
- Agita tubos Heidolph
- Congelador < -20°C
- Refrigerador ≤ 10°C
- Sonda de Temperatura calibrada en rango 70-110°C y con resolución de 1°C.
- Phmetro Metler Toledo.
- Sistema HPLC–MS/MS con espectrómetro de masas tándem de triple cuadrupolo (QqQ-MS), que consiste en un inyector automático Finnigan Surveyor, una bomba binaria LC y un detector de triple cuadrupolo TSQ Quantum Ultra (Thermofisher Scientific®, Bremen, Alemania)
- Sistema UHPLC (HR-Orbitrap-MS), con espectrómetro de masas de alta resolución, equipado con inyector automático, bomba modelo Accela, una interfaz de ionización por electro espray (HESI-II) y detector de masas Orbitrap MS de una etapa (Exactive ™, Thermofisher Scientific®, Bremen, Alemania).

3.2. Toma de muestras y conservación

3.2.1. Muestras de orina

Las muestras de orina analizadas en la presente Tesis fueron tomadas en el contexto del proyecto "*Bettermilk*" que tiene como principal objetivo la estimación de la exposición de niños lactantes alimentados con leche materna a contaminantes prioritarios, y a contaminantes emergentes. También tiene como objetivo realizar una estimación de la exposición a estos contaminantes en la madre, mediante el análisis de biomarcadores de exposición en orina y pelo.

El protocolo de muestreo, transporte y almacenamiento de las muestras de orina se establecieron de acuerdo con las guías internacionales del grupo de expertos de apoyo en materia de protocolos para la biomonitorización en Europa (ETSBEP 2015). Las muestras utilizadas corresponden a la primera orina de la mañana de madres lactantes, las muestras de orina se tomaron a las 2, 5 y 8 semanas después del parto. Las muestras se mantuvieron refrigeradas a 4°C y se transportaron al laboratorio dentro de las dos horas siguientes. Una vez recibidas, cada muestra se dividió en alícuotas de 10 ml y se congelaron a <-70ºC en el biobanco hasta su análisis.

3.2.2. Muestras de utensilios de poliamida y envases

Los envases destinados a contener alimentos fueron suministrados por dos Industrias de la Comunidad Valenciana durante el año 2016: Tetrabriks destinados a contener zumo (con pH <4.5) y zumo con leche (con pH> 4,5), además de bolsas destinadas a contener postres lácteos infantiles y bolsas para contener mosto de fruta (con pH> 4,5).

Los utensilios de cocina de poliamida fueron recogidos por las autoridades sanitarias en puntos de consumo de la Comunidad Valenciana dentro del Plan de vigilancia en seguridad alimentaria (VISA). Las muestras se conservaron a temperatura ambiente y resguardadas de la luz hasta su análisis.

3.3. Tratamiento de muestras

En la presente Tesis se han utilizado tres estrategias de preparación de muestras correspondientes a cada una de las combinaciones matriz-analitos (Ver figura 8).

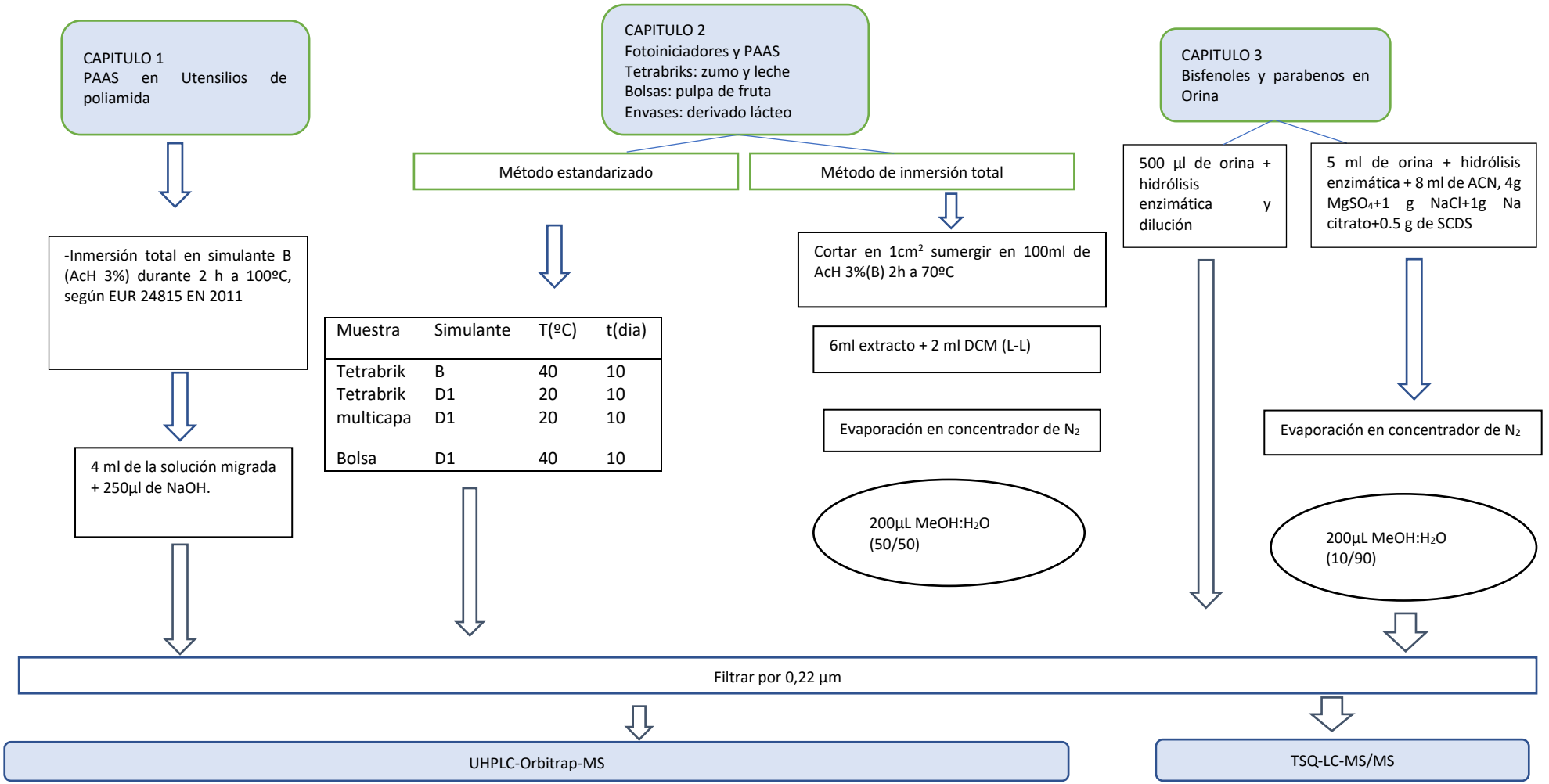
a) Para la extracción de PAAS en utensilios de poliamida se ha utilizado el método estandarizado descrito en la guía europea EUR 24815 EN 2011

b) Para la extracción de fotoiniciadores y PAAS en distintos envases y embalajes, se ha utilizado el método estandarizado cuyas condiciones de migración figuran en el reglamento

de la UE 10/2011, en el que los envases y muestras se someten a migración por inmersión total o por contacto con simulante, de la superficie del material sujeta a entrar en contacto con el alimento, según tablas 2, 3 y 4 detalladas en la introducción.

c) En el caso de las muestras de orina humana su tratamiento se realizó de dos formas: la primera consistió en una simple dilución tras la hidrólisis enzimática de la orina, y en la segunda se realizó una extracción con QUECHERS sin purificación. Se compararon ambos métodos en términos de exactitud y precisión.

En todos los métodos de preparación de muestra se han evitado las etapas de purificación con objeto de obtener métodos genéricos que puedan aprovechar el potencial del LC-HRMS para el análisis retrospectivo y la búsqueda de sustancias desconocidas. Al mismo tiempo se minimiza el consumo de disolventes y el tiempo de preparación de la muestra.



B: AcH 3%: Ácido acético 3%, D1: Etanol al 50%, DCM: diclorometano, L-L: líquido-liquido, Nacitrato: citrato sódico, SCDS: citrato disódico sesquidihrato.

Figura 8. Métodos de preparación/extracción de muestra utilizados

3.4. Separación y detección analítica

En todas las metodologías desarrolladas en la presente Tesis Doctoral, la separación cromatográfica tiene lugar mediante cromatografía liquida, empleando columnas analíticas de tamaño de partícula inferior o igual a 2.6 μm de diámetro (UHPLC), que consiguen una buena resolución cromatográfica sin producir una presión excesiva en el sistema.

El sistema de detección empleado en los métodos para PAAS y fotoiniciadores en utensilios de poliamida y distintos tipos de envases y embalajes es la espectrometría de masas de alta resolución con analizador Orbitrap (HR-Orbitrap-MS).

En el caso de la determinación de bisfenoles y parabenos en orinas se ha utilizado un analizador de masa de triple cuadrupolo, trabajando en masas en tándem (MS/MS).

En ambos sistemas, HPLC-MS/MS (TSQ Quantum) y HR-Orbitrap-MS, la interfase entre el HPLC y el analizador de masas está compuesta por la fuente de ionización, que permite la generación de iones tanto por electro nebulización (ESI), como mediante ionización química a presión atmosférica (APCI, Atmospheric Pressure Chemical Ionization). El proceso de ionización mediante ESI favorece el paso de iones formados previamente en disolución a iones en fase gaseosa y es el más utilizado en la cromatografía líquida acoplada a la espectrometría de masas. Sin embargo, en el caso de la APCI se favorece el paso de los iones en fase gaseosa ionizados a presión atmosférica. Los iones generados en esta interfase se introducen en el analizador.

Del enfoque analítico utilizado para la determinación de bisfenoles y parabenos en orinas HPLC-MS/MS (TSQ Quantum), cabe destacar la sensibilidad de estos equipos. Con ellos los límites de detección pueden llegar a ser muy bajos, en el rango de 0.03 $ng \cdot mL^{-1}$ a 0.06 $ng \cdot mL^{-1}$.

El uso de HRMS, que es el utilizado en la determinación de aminas y fotoiniciadores en materiales en contacto con alimentos, se debe principalmente a las ventajas de utilizar el modo de adquisición de barrido completo con alta sensibilidad combinada con alta capacidad de resolución (> 20.000 FWHM) y medición de la masa exacta (< 5 ppm), lo que permite una elevada selectividad y el posterior análisis retrospectivo.

Actualmente, los equipos HRMS de mayor uso en los laboratorios analíticos utilizan la tecnología de tiempo de vuelo (TOF) o la tecnología Orbitrap, ésta última utilizada en la

presente tesis. Estos equipos permiten combinar *target* análisis (análisis dirigido*)* y *"suspect screening"* (cribado de sospechosos) (Aceña et al. 2015, Núñez et al. 2012). Mediante el análisis no dirigido, todo m/z dentro del rango definido se registra durante todo el análisis, por lo que se guardan los datos del espectro completo. La característica principal del *"suspect screening"* (cribado de sospechosos) es que el conjunto de datos del espectro completo permite el análisis retrospectivo de la muestra incluso años después de la adquisición de datos.

En los capítulos 1 y 2 de la presente tesis se ha utilizado HRMS-Orbitrap con el objetivo de realizar el análisis retrospectivo y el "suspect screening" en materiales en contacto con alimentos.

Los detalles del método cromatográfico y de detección utilizados en la determinación de las moléculas de interés en los métodos desarrollados en la presente tesis (Capítulos 1-4) se detallan en la tabla 13.

Tabla 13. Métodos cromatográficos, detección y softwares utilizados

	CAPITULO 1 (Utensilios de poliamida)	**CAPITULO 2 (Tetrabriks, bolsas y envases)**	**CAPITULO 3 / 4 (Orina)**
Compuestos	Aminas aromáticas primarias	Fotoiniciadores y aminas aromáticas primarias	Bisfenol A, F, S y parabenos
Cromatografía	UHPLC	UHPLC	HPLC
Analizador	HRMS	HRMS	QqQ (MS/MS)
Columna cromatográfica	Phenyl hexil C18 (100 x2.1 mm) 2.6 µm	Hypersild gold aQ (100 x2.1 mm) 1.9 µm	Luna C18 (150 x2 mm) 5 µm
Modo adquisición	Full scan 50-800 Da ESI + sin/con HCD (20 eV)	Full scan 65-500 Da - ESI + sin/con HCD (20 eV) en el análisis target - ESI +/- sin HCD en el postarget	- APCI (-) Modo MS/MS - ESI (+/-) Modo MS/MS
Eluyentes	A: H_2O B: MeOH	A: H_2O con 0.1 % HCOOH B: MeOH con 0.1 % HCOOH	APCI, A: H_2O; B: MeOH ESI, A: H_2O con 5Mm de acetato amónico; B: MeOH
Software	Xcalibur 2.0(1) Trace Finder 3.0(2) MINITAB Release 14(3) MassFrontier(4)	Xcalibur 2.0(1) Trace Finder 3.1(2) MINITAB Release 14(3) MassFrontier(4)	Xcalibur 2.0(1) Trace Finder 3.2(2) MINITAB Release 14(3) MassFrontier(4)

HCD: Disociación inducida por colisión de alta energía, (1) y (2) software para el control instrumental, procesado y tratamiento de datos, (3) software para el diseño de experimentos y estadística, (4) software para la predicción de fragmentos y masas.

3.5. Criterios de identificación y confirmación

La correcta identificación y confirmación de las sustancias analizadas es una parte de los criterios de calidad exigible a los métodos analíticos. A continuación, se describen los criterios de identificación y confirmación empleados en los distintos trabajos de esta Tesis Doctoral. Todos los criterios de identificación y confirmación siguen las directrices de la guía SANTE/11813/2017.

3.5.1. Criterios de identificación y confirmación para PAAs y fotoiniciadores por UHPLC-HRMS

Para la identificación de los analitos, se siguen los siguientes criterios:

- Desviación de masa del ion molecular < 5 ppm respecto a la masa teórica del ion molecular
- Desviación de masa del fragmento < 5 ppm respecto a la masa teórica del fragmento
- Perfil isotópico similar al teórico con una tolerancia del 30%.
- El tiempo de retención de la muestra debe ser ± 0.1 min al tiempo de retención del patrón.

3.5.2. Criterios de identificación y confirmación para bisfenoles y parabenos por LC-MS/MS

Para la identificación y confirmación de las moléculas, se siguen los siguientes criterios:

- Detección de dos transiciones SRM por analito
- La diferencia en el tiempo de retención entre la muestra y el patrón no puede ser superior a 2.5 %.
- La diferencia de abundancia relativa de las transiciones en las muestras no puede ser mayor al 20 % de la diferencia obtenida en los patrones.
- La señal/ruido (S/N) de las dos transiciones ha de ser mayor a 3.

3.5.3. Criterios de identificación y confirmación para análisis retrospectivo de plásticos por UHPLC-HRMS

El screening de sospechosos se realiza mediante herramientas automatizadas, usando el programa TraceFinder. Los parámetros empleados fueron los siguientes: a) para el ion molecular, un umbral de 10000, con una S/N de 5, y una desviación de masa exacta inferior a 5 ppm; b) para los fragmentos, un umbral mínimo de 5000 con una S/N de 5, y una desviación de masa exacta inferior a 5 ppm; c) para el perfil isotópico un porcentaje de *fit threshold* del 90 %, con una intensidad relativa permitida del 30 %, y una desviación de la exactitud de masa de 5 ppm.

Para la identificación de los analitos, se siguen los siguientes criterios:

- Desviación de masa del ion molecular < 5 ppm respecto a la masa teórica del ion molecular
- Desviación de masa del fragmento < 5 ppm respecto a la masa teórica del fragmento
- Perfil isotópico similar al teórico con una tolerancia del 30%.

Para la confirmación de los analitos se utilizan patrones, y se comparan los tiempos de retención. El tiempo de retención de la muestra debe ser ± 0.1 min al tiempo de retención del patrón.

3.6. Diseño del estudio de biomonitorización y evaluación del riesgo

Una de las estrategias analíticas desarrolladas en esta tesis se ha aplicado a las muestras de orina de madres lactantes del proyecto "*Bettermilk"* en la Comunidad Valenciana. En la recolección de muestra, se obtuvo información sobre las características personales, la dieta y el estilo de vida de las madres participantes a partir de cuestionarios.

3.6.1. Tratamiento estadístico

En el estudio sobre la biomonitorización humana de parabenos y bisfenoles (Capítulo 4) se aplicó un análisis estadístico con el fin de estudiar las posibles asociaciones entre los niveles de bisfenoles y parabenos en orina con las variables físicas, sociodemográficas, de dieta y cuidados personales, obtenidos mediante cuestionarios.

El análisis estadístico se realizó utilizando el software R (versión 3.4.0). Los niveles de parabenos y bisfenoles en la orina por debajo del límite de cuantificación se estimaron siguiendo el método de máxima verosimilitud descrito en (EFSA 2010). Este método se basa en el supuesto de que los datos se distribuyen de acuerdo con una determinada distribución paramétrica y estima los parámetros de esta distribución de modo que se maximice la probabilidad de obtener la respuesta de la muestra observada. Se asumió una distribución log-normal para los niveles de parabenos y bisfenoles en la orina.

En primer lugar, se realizó un análisis descriptivo de todas las variables. Las variables cualitativas fueron descritas por frecuencias absolutas y porcentajes. Las variables cuantitativas se resumieron por su mediana y rango. Además, los niveles de parabenos y bisfenoles en la orina se resumieron calculando los percentiles mínimos, 25, 50, 75 y 95, medias aritméticas y geométricas, desviación máxima y estándar. Se utilizó el test de correlación de Spearman para estudiar la correlación entre los niveles de los diferentes tipos de parabenos y bisfenoles en la orina.

Se consideró la transformación logarítmica de los niveles de parabenos y bisfenoles para obtener la normalidad de las variables de respuesta y a partir de ésta, se construyeron modelos de regresión lineal simple y múltiple. La relación de la exposición a parabenos y bisfenoles con posibles determinantes (covariables y factores de confusión) se estudió mediante estas técnicas de regresión. Los factores de confusión son variables definidas a priori y que están relacionadas con el biomarcador (edad, consumo de alimentos envasados, uso de cosméticos); las covariables son posibles determinantes cuya relación con los niveles de parabenos y bisfenoles se probó dentro de la población de estudio. Todos los factores de confusión y covariables se pusieron en el análisis y modelos como cuantitativo (parámetros antropométricos), semicuantitativo (dieta) y variables categóricas o cualitativas (uso de cosméticos).

Los modelos de regresión múltiple se construyeron teniendo en cuenta las variables con p-valor inferior 0.05 obtenidas de los modelos de regresión simple y siguiendo un procedimiento de selección de variable hacia atrás basado en el criterio de información Bayesiano (BIC) (EFSA 2010).

Para que los resultados de los modelos de regresión puedan considerarse válidos, los residuos del modelo (diferencia entre los niveles de contaminantes observados y los predichos por el modelo) deben de verificar una serie de hipótesis: normalidad, media 0, varianza constante e independencia.

Habitualmente se consideran transformaciones de las variables respuesta los niveles de contaminantes que ayudan a que los residuos sigan una distribución normal. En todos los casos, MP, EP, PP y BPA se verifican las hipótesis de los residuos de los modelos todos los compuestos siguen una distribución logarítmico normal.

3.6.2. Cálculo de la ingesta diaria estimada y del índice de riesgo.

Para el cálculo de la ingesta diaria estimada (IDE) basado en datos de las concentraciones biomonitorizadas, se aplica un modelo toxicocinético comúnmente utilizado (Ecuación (1)) para convertir las mediciones urinarias de biomarcadores en IDE.

$$IDE = \frac{C\ (^{\mu g}/_{l}) \cdot Vorina(^{l}/_{dia})}{F \cdot Peso(kg)}$$

(Ecuación 1)

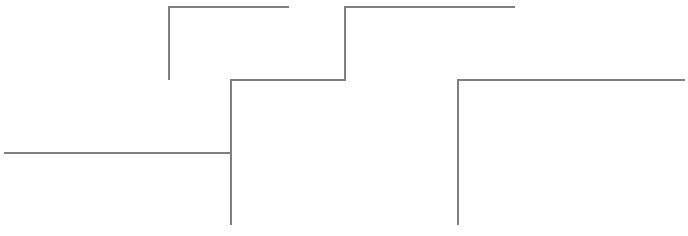
C, concentración de bisfenoles o parabenos; Vorina, el volumen urinario total excretado en 24 h; F, factor de excreción urinaria del compuesto; Peso, el peso corporal de referencia. La concentración considerada corresponde al percentil 95 del estudio realizado. El volumen de orina para adultos considerado fue de 1.50 L (Dirtu et al. 2013) y el peso corporal de 70 kg (WHO 2006). Al no disponer de factor de excreción urinario en la bibliografía para los parabenos, se consideró 1 cómo valor optimista y 0.25 cómo pesimista.

El HQ es la relación entre la ingesta diaria estimada y el nivel en el que no se esperan efectos adversos, como una IDA (ingesta diaria admisible). Si HQ > 1, la concentración total (o dosis) de los componentes de la mezcla excede el nivel considerado aceptable (EFSA 2013)

$$HQ = \frac{IDE\ calculado}{IDA\ (teórico)}$$

(Ecuación 2)

4. Resultados



Capítulo 1

4.1. Capítulo 1. Análisis de aminas aromáticas primarias combinado con un método de cribado amplio de sustancias que pueden migrar desde utensilios de cocina de poliamida por UHPLC-HRMS

4.1.1. Resumen

Las aminas aromáticas primarias (PAAS) aparecen en los materiales en contacto con los alimentos debido a su utilización en la fabricación de múltiples polímeros, cómo los poliuretanos, que a su vez se emplean en los envases para alimentos, y en los utensilios de cocina de poliamida (Trier et al. 2010).

Las aminas cuando migran pueden permanecer inalteradas o, debido a su alta reactividad, formar nuevos compuestos con otras sustancias. Estas nuevas sustancias formadas son completamente desconocidas y no están evaluadas toxicológicamente, pudiendo representar un riesgo para la salud de los consumidores, con lo que deberían ser identificadas y cuantificadas (Pezo et al. 2012).

Cada año se producen notificaciones y alertas alimentarias por incumplimiento de requisitos en artículos de plásticos procedentes de países que exportan a la Unión Europea. La última notificación que consta en el sistema de alertas de la Comisión Europea (RASFF) es de abril del 2019 en utensilios de poliamida en Italia. Estos incumplimientos se han relacionado con la liberación de aminas aromáticas primarias y formaldehido a los alimentos a partir de envases de plástico de compuestos de poliamida y melamina, respectivamente. Por ello, la Unión Europea ha puesto en marcha numerosas iniciativas y ha establecido medidas específicas para minimizar los riesgos derivados de estos materiales plásticos.

Las citadas medidas específicas se recogen en el Reglamento EU 284/2011 de la Comisión, de 22 de marzo de 2011, por el que se establecen condiciones específicas y procedimientos detallados para la importación de artículos plásticos de poliamida y melamina. Además el Reglamento EU 10/2011, establece los límites de migración para sustancias procedentes de plástico a alimentos, concretamente este límite es de 0.01 mg de sustancias por kilogramos de alimento o simulante y se aplica a la suma de aminas aromáticas primarias (ANL, 2-4 TDA, 2-6 TDA, 4-4 MDA, 1-5 DAN, m-PDA, 3-3 DCB

y 4-4' DPE), por lo que, es necesario un control oficial para verificar el cumplimiento de la legislación vigente.

En la literatura revisada se detecta la falta de métodos multianalito para la determinación de las aminas. La mayoría de los métodos descritos son procedimientos largos, tediosos que analizan las aminas, particularmente la Anilina, de forma individual. Brede et al 2003, aplicó tres técnicas distintas HPLC-UV, GC-MS y métodos espectrofotométricos para la determinación de la Anilina en utensilios de poliamida. Posteriormente, muchos métodos para la determinación de PAAs han sido publicados empleando técnicas como: GC/MS con derivatización (Kawakami et al. 2010) y electroforesis capilar (Wang et al. 2009), (García Lavandeira et al. 2010).

Por otro lado Schubert et al. (2011), McCall et al. (2012) utilizaron LC acoplado a una espectrometría de masas en tándem (MS). Se necesitan métodos analíticos para la determinación simultánea de aminas que faciliten el análisis en los laboratorios de control. Esta necesidad de métodos analíticos cada vez más sensibles, que abarquen gran número de compuestos y que además permitan el análisis retrospectivo es lo que lleva al desarrollo de una metodología genérica por UHPLC-HRMS.

En este capítulo (artículo 1), el principal objetivo es el desarrollo de una estrategia analítica adecuada para el análisis de aminas aromáticas primarias procedentes de utensilios de cocina de poliamida empleando la cromatografía líquida acoplada a espectrometría de masas de alta resolución (UHPLC-HRMS). Esta estrategia está basada en dos etapas diferenciadas: i) análisis cuantitativo de compuestos conocidos (PAAs) (Target análisis o análisis dirigido) y, ii) análisis "*suspect screening*" (cribado de compuestos sospechosos) de contaminantes procedentes de los plásticos a partir de la creación de una base de datos de 77 compuestos tales como, derivados epóxidos, fotoiniciadores, plastificantes, NIAS y ftalatos (figura 9).

En el presente trabajo, la extracción de aminas primarias de los utensilios de poliamida se realizó conforme al Reglamento 10/2011, por migración estandarizada. Las condiciones de temperatura, tiempo de contacto y simulante del ensayo fueron de 2 horas a 100 ºC y simulante B (Ácido Acético al 3%). La metodología analítica ha sido aplicada a 10 muestras de utensilios de cocina de uso repetido procedentes de distintos puntos de consumo de la Comunidad Valenciana.

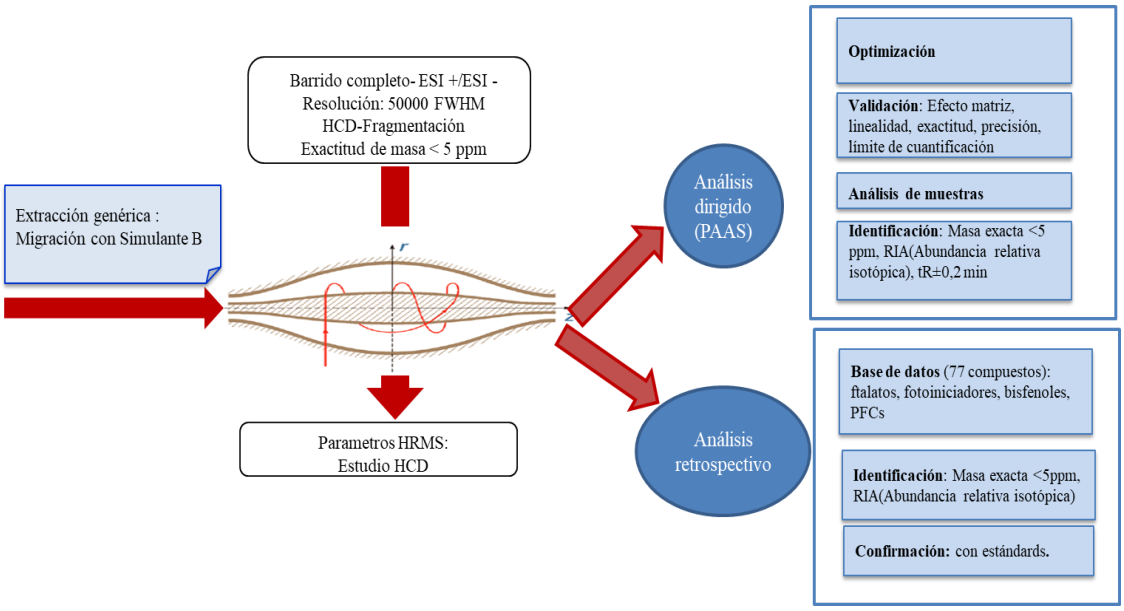


Figura 9. Estrategia de análisis de aminas primarias (PAAS) en utensilios de poliamida.

4.1.2. Discusión de resultados

a. Optimización de los parámetros de la fuente de ionización (ESI)

La separación cromatográfica se optimizó con el estudio de cuatro columnas cromatográficas C18 y varios eluyentes. Se seleccionó la columna *Phenyl hexyl* (100 x 2,1mm) de 2.6 µm, con un gradiente binario de metanol agua que proporciona una adecuada separación y formas de pico de los analitos de interés, especialmente de los isómeros 2,4 y 2, 6 TDA.

Como resultado del diseño estadístico de experimentos se establecieron los parámetros más relevantes de la fuente de ionización. De los siete parámetros seleccionados para la optimización, la presión del gas auxiliar (AG), el voltaje del capilar (CV) y voltaje del skimmer (SKV), resultaron parámetros no significativos como resultado del cribado mediante un diseño de experimentos de *Plackett-Burman* (PB). Los valores para estos parámetros se establecieron en el punto medio del rango estudiado. Mediante diseño de compuesto central (CCD) se obtuvieron los valores óptimos para los cuatro factores significativos: presión de gas envolvente (SGP), temperatura del capilar (CT), el voltaje de espray (SV) y la temperatura de calentamiento (HT). En la tabla 14 se muestran los resultados óptimos obtenidos del diseño estadístico de experimentos para todos los parámetros estudiados.

Tabla 14. Valores óptimos de los parámetros de la fuente de ionización (ESI)

	PARÁMETRO						
	SGP (a.u.)	**AG (a.u.)**	**SV (kV)**	**CT (ºC)**	**CV (V)**	**SKV (kV)**	**HT (ºC)**
Rango estudiado	5-60	0-20	±(2-5)	100-450	20-70	1-50	100-500
Valor óptimo	**50**	10	**+4.4**	**123**	45	23	**500**

b. Estudio de fragmentación HCD

El estudio del impacto de diferentes energías de colisión (0, 10, 15, 20, 25 y 30 eV) en la respuesta de fragmentos e ion molecular se realizó con el fin de optimizar la señal de ambos iones. En el caso del 2,6 TDA la energía óptima de fragmentación es 17 eV. Por el contrario, en la mayoría de los casos como por ejemplo 1,5 DAN, ANL, m-PDA esta energía resulta demasiado baja (figura 10).

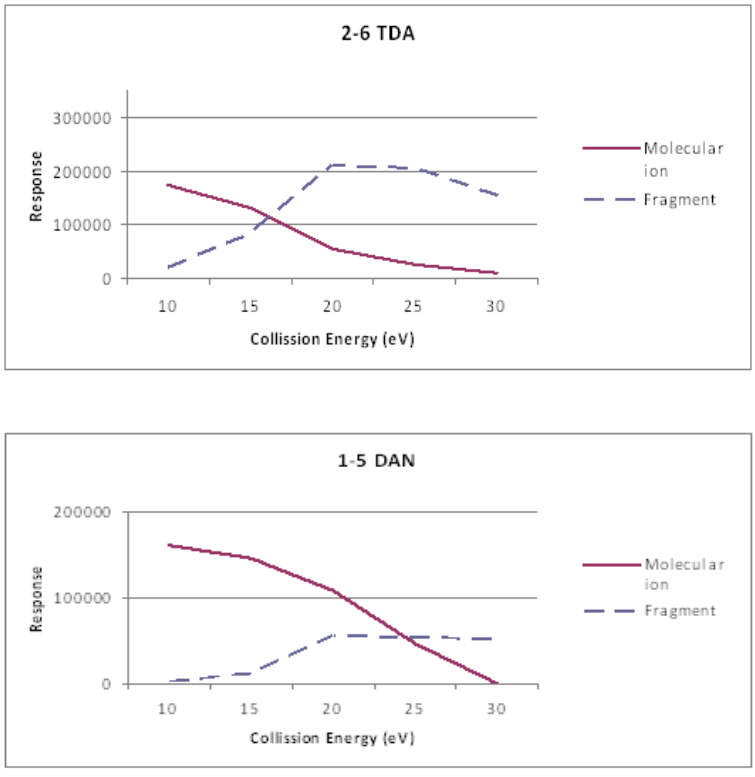


Figura 10. Fragmentación HCD para 2,6 TDA y 1,5 DAN a las diferentes energías de colisión (10-30eV)

Se estableció una energía óptima de colisión de 20 eV de manera que la respuesta en área para la mayoría de los analitos tanto para el ion molecular como el fragmento representativo fuera la más favorable.

c. Efecto matriz y validación

En la mayoría de los casos se observó una supresión iónica. Las aminas con mayor supresión iónica fueron la anilina y la m-PDA, con valores entre el 20 y 50%. Las aminas 4,4'DPE, 2,4-TDA, 2,6 TDA y 1,5DAN, presentaron un efecto matriz (ME) moderado con valores en el rango de 60-80%. Los únicos analitos que no presentaron efecto matriz fueron los 4,4' MDA y 3,3' DMB. A la vista de estos resultados, puede resultar adecuada una etapa de purificación adicional a la extracción. Sin embargo, la inclusión de esta etapa va en perjuicio de una extracción genérica necesaria para el análisis retrospectivo de un amplio número de compuestos. Por otro lado, este efecto matriz puede ser compensado y corregido mediante otras vías como el uso de estándares internos o calibración sobre matriz. En este trabajo, dichos métodos de corrección fueron utilizados para la obtención satisfactoria de resultados cuantitativos. Las recuperaciones para la mayoría de los analitos, incluso para los compuestos con ME alto, varían en un rango de 78-120% con coeficientes de variación < 15%, para los tres niveles validados (límite de cuantificación, intermedio y alto). Los resultados para cada amina se detallan en el artículo científico.

d. Análisis target y "suspect screening" de muestras reales

Previo a la puesta a punto del método cromatográfico se construyó una base de datos amplia de 77 analitos objeto de investigación. En esta base de datos se incluyó, para cada compuesto, la composición elemental, el modo de ionización (ESI^+, ESI^- o ambas) y la masa exacta del ion diagnóstico, así como de sus posibles fragmentos, con 4 cifras decimales.

El análisis target (dirigido) fue aplicado satisfactoriamente a 10 muestras reales, en las que fueron identificadas, cuantificadas y confirmadas 6 aminas en el rango de concentración siguiente: 2,6-TDA (9- 12 µg/kg), 2,4-TDA (2.7- 4.7 µg/kg), 4,4'- MDA (2-19.7 µg/kg), 3,3'DMB (2.7-49 µg/kg), m-PDA (2 µg/kg) y ANL (2.5-284 µg/kg). Las figuras 11 y 12, corresponden a la confirmación de dos de las aminas encontradas en dos muestras distintas.

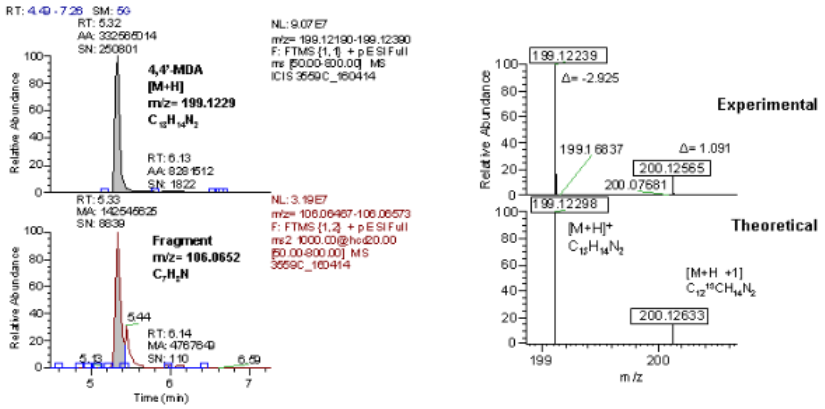


Figura 11. Confirmación mediante los fragmentos característicos y el patrón isotópico teórico y experimental de 4,4' MDA en un cucharón de poliamida.

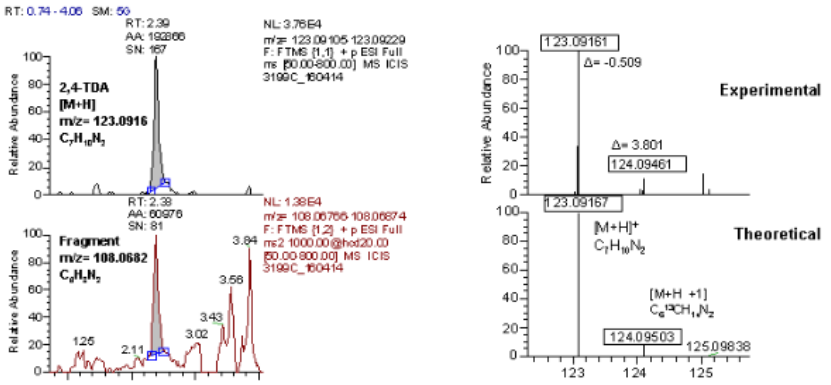


Figura 12. Confirmación mediante los fragmentos característicos y el patrón isotópico teórico y experimental de 2,4 TDA en una paleta de poliamida

Por otro lado, se realizó el cribado de todas las muestras mediante comparación con la base de datos creada de 77 compuestos ("suspect screening"). Dos ftalatos, DnBP y DEHP, se detectaron en el análisis *postarget*, la confirmación completa se realizó posteriormente tras la adquisición de los estándares.

4.1.3. Conclusiones

- La estrategia analítica desarrollada combina el análisis cuantitativo de 8 PAAs en utensilios de poliamida (*target*) con un cribado retrospectivo de distintas familias de compuestos procedentes de los materiales en contacto con los alimentos (envasado), mediante el uso de UHPLC-HRMS.

- El método cuantitativo presenta límites de cuantificación menores de 2µg kg^{-1} para la mayoría de los analitos, con recuperaciones entre el 60 y el 120 % y precisiones menores del 15%, por lo que este método resulta útil para el control oficial.
- El potencial analítico de la alta resolución, la masa exacta y la adquisición en barrido completo con y sin fragmentación, permite la comparación con bases de datos para la identificación inicial de posibles contaminantes no incluidos *a priori* en el método (análisis retrospectivo).
- La aplicación del método desarrollado en muestras reales combinando el análisis *target* y el *"suspect screening"* hacen de esta estrategia una prometedora herramienta para estudios de exposición a contaminantes procedentes del envasado y de los materiales.

4.1.4. Artículo 2. Target analysis of primary aromatic amines combined with a comprehensive screening of migrating substances in kitchen utensils by liquid chromatography-high resolution mass spectrometry

Talanta 138 (2015) 290–297

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Target analysis of primary aromatic amines combined with a comprehensive screening of migrating substances in kitchen utensils by liquid chromatography–high resolution mass spectrometry

Yovana Sanchis [a,b], Clara Coscollà [a,b], Marta Roca [a,b], Vicent Yusà [a,b,c,*]

[a] Foundation for the Promotion of the Health and Biomedical Research in the Valencia Region (FISABIO-Public Health), 21, Avenida Catalunya, 46020 Valencia, Spain
[b] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain
[c] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain

ARTICLE INFO

Article history:
Received 16 December 2014
Received in revised form
9 March 2015
Accepted 15 March 2015
Available online 23 March 2015

Keywords:
Primary aromatic amines
High-resolution mass spectrometry
Orbitrap
Experiment design
Post-run target
Accurate mass

ABSTRACT

An analytical strategy including both the quantitative target analysis of 8 regulated primary aromatic amines (PAAs), as well as a comprehensive post-run target screening of 77 migrating substances, was developed for nylon utensils, using liquid chromatography–orbitrap-high resolution mass spectrometry (LC–HRMS) operating in full scan mode. The accurate mass data were acquired with a resolving power of 50,000 FWHM (scan speed, 2 Hz), and by alternating two acquisition events, ESI+ with and without fragmentation. The target method was validated after statistical optimization of the main ionization and fragmentation parameters. The quantitative method presented appropriate performance to be used in official monitoring with recoveries ranging from 78% to 112%, precision in terms of Relative Standard Deviation (RSD) was less than 15%, and the limits of quantification were between 2 and 2.5 μg kg^{-1}. For post-target screening, a customized theoretical database was built for food contact material migrants, including bisphenols, phthalates, and other amines. For identification purposes, accurate exact mass (< 5 ppm) and some diagnostic ions including fragments were used. The strategy was applied to 10 real samples collected from different retailers in the Valencian Region (Spain) during 2014. Six out of eight target PAAs were detected in at least one sample in the target analysis. The most frequently detected compounds were 4,4′-methylenedianiline and aniline, with concentrations ranging from 2.4 to 19,715 μg kg^{-1} and 2.5 to 283 μg kg^{-1}, respectively. Two phthalates were identified and confirmed in the post-run target screening analysis.

1. Introduction

Primary aromatic amines (PAAs) appear in food products mainly from food contact materials (FCMs). These amines are formed by hydrolysis of aromatic isocyanates in polyurethane adhesives, and by degradation of azodyes used as colourants in nylon kitchen utensils and other plastic materials [1]. Evidence suggests that some PAAs are carcinogenic pollutants [2], consequently their migration into foodstuff is subjected to regulations and restrictions. The European Union Regulation 10/2011 has established a migration limit of 0.01 mg kg^{-1} for the sum of the regulated primary amines [3].

Brede et al. [1] identified the polyamide cooking utensils as a common source of PAAs. Likewise, Mortensen et al. [4] also detected the migration of several PAAs, especially aniline (ANL) and 4,4′-methylenedianiline (MDA) from black polyamide cooking utensils, which exceeded the limits permitted by the EU legislation. From these early studies onwards, numerous alerts have been issued by the rapid alert system for food and feed (RASFF) for excessive concentrations of PAAs migrating from FCMs [5].

The need to carry out a continuous surveillance and the frequently detected violations of the migration limits has led to the development of different analytical methods. The most commonly used technique for determining PAAs has been gas chromatography coupled to mass spectrometry (GC–MS), after derivatization [1]. Additionally, liquid chromatography–tandem mass spectrometry (LC–MS/MS) [3,6–8] has currently become a very sensitive and selective analytical tool for the quantitative determination of these polar compounds. However, LC–MS/MS presents some limitations such as the impossibility to conduct a retrospective analysis of the

* Corresponding author at: Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain. Tel.: +34 961925865; fax: +34 961925704.
E-mail address: yusa_vic@gva.es (V. Yusà).

http://dx.doi.org/10.1016/j.talanta.2015.03.026
0039-9140/

samples for the post-target identification of other relevant compounds not included in the initial scope.

In some fields such as pesticide and veterinary drug analysis, LC coupled to high-resolution mass spectrometry (HRMS), using Orbitrap and time-of-flight (TOF) analysers has increasingly become more popular [11–13] owing to its capacity of using the full-scan acquisition mode with high sensitivity, combined with high resolving power (> 50,000 FWHM) and accurate mass measurements (1–5 ppm). This powerful tool enables the development of both a quantitative analysis of the priority regulated substances (target analysis) and a post-target screening of suspected compounds. Mattarozzi et al. [9] have recently developed and validated an innovative LC–HRMS method for the comprehensive determination of PAAs in plastic multilayer food-packaging. However, the authors do not include the retrospective analysis of other possible substances migrating from the plastic material.

In the present study we have developed an analytical strategy that combined the quantitative target analysis of the 8 regulated PAAs, with the post-target screening (identification) of 77 specific migration substances from nylon kitchen utensils, using a standardized extraction protocol with acetic acid (simulant) and direct injection into an LC–HRMS without clean-up. The analytical strategy was applied to samples collected in an official monitoring programme in the Valencia Region, Spain.

2. Experimental

2.1. Chemicals and reagents

High purity standard amines, 2,6-toluenediamine (98%) (2,6-TDA); 2,4 toluenediamine (98%) (2,4-TDA); aniline (98%) (ANL); 1,5-naphthalenediamine (99%) (1,5-DAN); 1,3-phenylenediamine (99%) (m-PDA); 4,4′-methylenedianiline (98.5%) (4,4′-MDA); 4,4′-oxydianilina (99%) (4,4′-DPE); and 3,3′-dimethylbenzidine (98%) (3,3′-DMB) were supplied by Sigma Aldrich. The food simulant was 3% acetic acid in water (w/v) and its density was conventionally set to 1.0 g cm^{-3}.

Individual stock standards were prepared weighting 25 mg of pure standard using a 5-decimal analytical balance and dissolving it in methanol. Mix working solutions at 5 μg mL^{-1} were prepared with methanol and at 100 ng mL^{-1} were prepared with acetic acid (AcH, 3%). Calibration solutions (1.5 or 2, 5, 10, 15 and 20 ng mL^{-1}) were prepared by adding variable volumes of the mix working solutions to the simulant (3% AcH in water, w/v).

HPLC-grade methanol was supplied by Scharlau (Barcelona, Spain). HPLC-grade water was purchased from Merck (Darmstadt, Germany). Acetic acid (Reag. Ph. Eur.) and ammonium hydroxide solution 25% were provided by Panreac (Barcelona, Spain).

Statistical data manipulation and numerical analysis of data resulting from the experimental design were carried out using the statistical package MINITAB for Windows, Release 14 (Minitab Inc., Birmingham, UK).

2.2. Samples

Ten black and grey nylon kitchen utensils were sampled from different retailers by the Food Safety Department of the Valencian Region (Spain) during 2014. Each sample consisted of 3 replicated for the migration test and another one for the surface calculation.

2.3. Sample preparation

The migration test was carried out following the European Standard EN 13130-1:2004 [10,14]. In short, each sample was placed in a beaker which was in turn filled with a volume of simulant (AcH, 3%) enough to cover the piece of utensil used for extraction. The beaker was covered with aluminium foil to avoid light exposure, and transferred to a preheated boiler. The top of the beaker was also covered with aluminium foil to reduce simulant loss by evaporation. After 2 h at 100 °C, test specimens were removed from the simulant and it was cooled down to room temperature. Then, the extract was placed in an Erlenmeyer flask with a glass stopper and stored at 4 °C until analysis. The volume of the acetic acid added into the beaker before and after the extraction was measured and recorded for the expression of results.

1 ml of extract was neutralized with 250 μl of ammonium hydroxide solution and filtered using a 0.45 μm microfiber filter (Whatman, GE Healthcare, Little Chalfont, UK) before injection in the LC–HRMS. Each sample was analysed in triplicate.

2.4. LC–HRMS

The chromatographic separation was performed on an Accela liquid chromatography UHPLC system equipped with a Phenyl hexil column (100 mm × 2.1 mm, 2.1 μm), both from ThermoFisher Scientific (Bremen, Germany). The flow rate used was 400 μl min^{-1} and the injection volume was 10 μl. Separations were performed using a binary gradient. The mobile phase was composed of H_2O (A) and methanol (B) and the binary gradient conditions were as follows: 0–8 min, linear from 2% to 50% B; 8–10 min, linear from 50% to 80% B; 10–11 min, linear from 80% to 95% B; 11–13 min isocratic 95% B; 13–15 min, linear from 95% to 2%. The total run time was 17 min.

Mass spectrometric analysis was performed on a single-stage Orbitrap MS (Exactive™, Thermofisher Scientific, Bremen, Germany). The system was equipped with a heated electrospray ionization interface (HESI-II). The detection was carried out in positive ionization mode (ESI+) using the following optimized operational parameters: spray voltage, 4.4 kV; sheath gas (N_2, > 95%), 50 arbitrary units (a.u.); skimmer voltage, 50 V; capillary voltage, 50 V; heater temperature, 500 °C; and capillary temperature, 123 °C. The mass spectra was acquired using two alternating acquisition functions (i) full scan MS without fragmentation, ESI+; mass resolving power=50,000 FWHM; scan range=80–800 Da; scan time=0.5 s (2 Hz); (ii) the same parameters but with full scan MS all ions fragmentation (the higher collision cell (HCD) was switched on at a collision energy of 20 eV). The automatic gain control (AGC) was set to 1×10^6 ions. The external mass calibration of the spectrometer was performed using a ready-to-use calibration mixtures (Mas Cal5 (+) from Supelco (USA). Data acquisition and processing was performed using Thermo Scientific TraceFinder™ software version 3.1. Extracted ion chromatograms (XIC) for individual compounds were reconstructed from the full-scan data with a mass tolerance of 5 ppm.

2.5. Analytical method validation and identification criteria

Samples were analysed under quality assurance protocols following the ISO 17025. Quality control procedures to check the method performance were implemented in each batch of samples, including reagent blanks, matrix blanks and spiked blank samples. Market samples were stored at −4 °C until analysis.

As there is no specific guideline prescribed for validation of methods concerning migrating substances from food contact materials, the quantitative method was validated using the SANCO/125771/2013 guideline [15], defining the following performance criteria: recoveries within 70–120%; repeatability RSD ≤ 20 and LOQ lower enough to be used for regulatory purposes [3]. There is no reference material available on primary amines in contact-food materials, so the accuracy of the method was carried out using a

spiked blank of migration solution at three concentration levels, corresponding to low (2 μg kg^{-1}), medium (12.5 μg kg^{-1}), and high (25 μg kg^{-1}), levels within the calibration range for each amine, with six replicates at each level. Precision was assessed as coefficient of variation (%), over 5 days.

The limit of quantification (LOQ) was defined as the lowest level of analyte that can be determined with acceptable precision and trueness (accuracy), following the Eurachem Guide [16] and SANCO/125771/2013 guideline [15]. ANOVA and Mandel's fitting tests were performed to check for linearity [17–19].

For the identification of analytes in the target quantitative method the following criteria were established: (i) mass accuracy of the molecular ion <5 ppm; (ii) mass accuracy of the fragment ion (HCD) <5 ppm and/or isotope pattern similar to the theoretical one; (iv) ion ratio similar to the standards with a relative tolerance of $\pm 30\%$, and (v) retention time similar to that of the calibration standard ± 0.2 min.

2.5.1. Database for post-run target screening analysis

A customized theoretical database containing 77 specific food-contact migrants was built grounded on the published literature [20]. Among the contaminants included in the database were phthalates, photoinitiators, bisphenols and perfluorinated compounds (Supplementary material, Table SI-1). For each substance, the screening database included the elemental composition and the theoretical accurate mass of the monitored molecular (quasi) ion. In this theoretical database no standards were used to get characteristic fragments [20-22]. Information about fragments was included when available in the literature, mainly from HRMS and QqQ (nominal mass) studies. When no fragments were found in the literature, those predicted by the software MassFrontier (version 7.0 from ThermoFisher) were included in the database. Likewise, the exact mass for all molecular and fragment ions was established and/or checked using both the MassFrontier and Xcalibur software (ThermoFisher).

To perform the screening and quantitative data analysis, the TraceFinder™ software (version 3.1, from ThermoFisher) was used. The identification and confirmation settings included a threshold override of 10,000, with S/N of 5 and a mass tolerance of 5 ppm for the molecular ion; an intensity threshold of 5000 and a mass tolerance of 5 ppm for the fragments. For the isotopic pattern a fit threshold of 90%, an allowed intensity deviation of 30%, and a mass deviation of 5 ppm were used. For quality control of the automated compound screening process made by the software, a few target blank and fortified samples were processed before and after the real samples.

3. Results and discussion

3.1. Chromatographic separation: HPLC study

In order to achieve an adequate chromatographic separation different columns and mobile phases were tested. For separation of individual PAAs we tested the following set of HPLC columns: Hypersil Gold aQ column 2.1 μm (ThermoFischer Scientific), Kinetex C18 2.6 μm (Phenomenex), Synergy Polar RP 2.5 μm (Phenomenex) and phenyl hexyl 2.1 μm (Thermofischer Scientific). The best separation was achieved on phenyl hexyl 2.1 μm, which is made of ether-linked phenyl with a polar end and which provides a good separation of polar and aromatic analytes such as PAAs (Table 1). Likewise a number of different mobile phases and additives were investigated. A general trend was the loss of signal intensity particularly for aniline and m-PDA when ammonium formiate or ammonium acetate was added. Addition of formic acid to mobile phase A resulted in faster elution of amines combined with insufficient separation. The best signal intensity and separation was achieved with water as eluent A and methanol as eluent B.

3.2. Optimization of ESI ion source settings: central composite design

The main factors affecting the response of analytes are ion source related. Although the Orbitrap mass spectrometer has an automated source tuning, better improvements in the analyte responses can be achieved using a statistical design of experiments (DoE) [18], mainly when a multiresidue method was needed. In view of the literature [23], the main factors affecting the ESI ion source efficiency are spray voltage (SP), sheath gas pressure (SGP), capillary temperature (CT) and heater temperature (HT).

A central composite design (CCD) [18] was used to obtain a more accurate optimization of these four parameters. This type of experimental design allows the building of the response surface, as well as finding the factor settings or operating conditions that maximize compound response. The design consisted of a two-level full factorial design 2^4 (16 cube points), 4 centre points in cube, 8 axial points and 2 centre points in axial. The 30 runs were randomized to provide protection against the effect of hidden variables. The values corresponding to every factor in each experiment and the responses for each analyte are shown in Table 3.

For each compound the analytical responses obtained were fitted into an equation that included second-order (curvature) and interaction terms. The model was validated using a regression analysis of variance (ANOVA). The next step was to select the factor settings that maximized the compound response. The factor settings that simultaneously maximized the responses of the eight compounds were selected using the "response optimiser" in the MINITAB software.

As we have multiple responses (one for each analyte), and as the response surfaces are different for each compound, it is necessary to

Table 1
Elemental composition, theoretical ion mass and fragment ion of the target compounds.

Compound	Elemental composition	Diagnostic ion	Theoretical monitored mass (*m/z*) ion (Da)	RT (min)	Fragment (*m/z*) ion/elemental composition
m-PDA	$C_6H_8N_2$	$[M+H]^+$	109.0760	1.30	92.04966 (C_6H_5N)
2,6-TDA	$C_7H_{10}N_2$	$[M+H]^+$	123.0916	1.99	108.06825 ($C_6H_8N_2$)
2,4-TDA	$C_7H_{10}N_2$	$[M+H]^+$	123.0916	2.43	108.06825 ($C_6H_8N_2$)
1,5-DAN	$C_{10}H_{10}N_2$	$[M+H]^+$	159.0916	3.61	143.07287 ($C_{10}H_9N$)
ANL	C_6H_7N	$[M+H]^+$	94.0651	2.69	76.0308 (C_6H_4)
4,4′-DPE	$C_{12}H_{12}N_2O$	$[M+H]^+$	201.1022	4.91	108.04470 (C_6H_8ON)
4,4′-MDA	$C_6H_4NH_2$	$[M+H]^+$	199.1229	5.35	106.06120 (C_6H_8N)
3,3′-DMB	$C_{14}H_{16}N_2$	$[M+H]^+$	213.1386	5.01	181.08027 ($C_{13}H_{11}N$)

find a factor setting that simultaneously maximizes the desirability for each response. It must be noticed that the desirability is 0.0 for the lowest values obtained in the CCD, increases as response values increase, and is 1.0 for the highest response obtained in the experiments. For this reason, we maximize a composite desirability that combined the individual desirability of all response variables into a single measure taking into account that all response variables are equally important. The optimized factor settings were spray voltage, 4.4 kV; sheath gas pressure, 50 a.u.; capillary temperature, 123 °C; and heater temperature, 500 °C, which provided a composite desirability of 0.624. As an example, Fig. 1 shows the response surface obtained by using the aforementioned model for some of the studied amines such as 2,6-TDA,1,5-DAN and 4,4′-MDA. The three-dimensional response surfaces show the effect of two independent variables on a given response, at a constant value of the other variable.

Table 2
Quality parameters for the target analysis.

Compounds	Levels						
	LOQ (μg/kg)	1.5 ng/ml[a]/ 2 μg/kg simulant		10 ng/ml[a]/ 12.5 μg/kg simulant		20 ng/ml[a]/25 μg/ kg simulant	
		Recovery (%)	CV (%)	Recovery (%)	CV (%)	Recovery (%)	CV (%)
m-PDA	2	112	15	96	6	99	6.5
2,6-TDA	2	121	6	104	5	101	2.19
2,4-TDA	2	117	11	99	9	107	7.29
1,5-DAN	2	102	13	102	4	100	4.23
4,4′-DPE	2	114	14	94	7	99	3
4,4′-MDA	2	91	12	99	3	102	2
ANL	2.5	78[b]	15[b]	79	15	93	15
3,3′-DMB	2.5	110[b]	11[b]	98	7	99	4

[a] Levels in ng/ml (concentration in vial).
[b] For ANL and 3,3′-DMB recoveries and variation coefficients (CV) are in 2.5 (μg/kg).

3.3. Study of HCD fragmentation

Fragments provide valuable information for analyte identification. The Orbitrap instrument incorporates a high energy collisional dissociation (HCD) Cell that consists of a straight multi-pole device mounted within a metal tube filled with N_2 as a collision gas. Ions generated in the source could be fragmented in the HCD generating spectra that should show comparable fragment ion formation to that of triple quadrupole analysers, albeit without any precursor selection (all ions fragmentation). In order to select the appropriate collision energy that provides the best response for both the characteristic ion of the target compounds (quasi-molecular ion) and their main fragment, a study on the impact of different collision energies (between 10 and 30 eV) on the responses of both ions was carried out.

After data analysis, a collision energy of 20 eV was selected for all compounds, which provided good sensitivity for both the molecular ions and their fragments. As an example, Fig. 2 shows the response obtained for some target amines when applying different collision energies

3.4. Matrix effect and analytical performance of the method for target analysis

In HPLC–MS, both in ESI or APCI ionization modes, the co-extractive matrix components can lead to a suppression or enhancement of the analyte signal as a result of the interferences in the ionization mechanism. The matrix effect (ME) can severely compromise quantitative analysis at trace levels, so the matrix

Table 3
Optimization of ESI ionization source settings: central composite design.

Run	Sheath gas (a.u.)	Spray voltage (kV)	Capillary temperature (°C)	Heater temperature (°C)	2,6-TDA (*m/z* 123)	2,4-TDA (*m/z* 123)	m-PDA (*m/z* 109)	1,5-DAN (*m/z* 159)	ANL (*m/z* 94)	4,4′-DPE (*m/z* 201)	4,4′-MDA (*m/z* 199)	3,3′-DMB (*m/z* 213)
1	33	6	275	300	175,244	562,451	75,388	1,150,250	2,21,050	1,199,608	1,112,402	1,990,042
2	60	4	275	300	175,052	577,675	75,379	1,419,900	12,286	1,533,032	1,534,256	2,138,065
3	5	4	275	300	0	35,239	8959	154,441	0	295,207	272,637	250,993
4	33	4	275	300	145,951	616,004	68,494	1,602,805	5458	1,872,889	1,704,882	2,675,038
5	33	4	100	300	232,858	838,918	101,002	2,149,896	22,896	1,826,532	1,675,851	2,037,339
6	33	4	275	300	156,629	601,711	64,795	1,642,030	11,833	1,885,581	1,744,434	2,731,018
7	33	4	275	500	140,965	73,982	66,462	2,258,572	11,014	3,676,737	3,945,988	5,494,274
8	33	4	275	100	139,492	297,000	50,135	621,127	0	687,893	740,235	1,342,505
9	33	4	450	300	4035	7328	10,029	126,905	10,944	344,931	404,943	5,339,726
10	33	1	275	300	92,972	179,400	43,209	494,666	0	225,903	169,121	419,267
11	46	2	363	200	27,736	34,592	31,069	293,457	0	424,047	437,049	964,123
12	19	2	363	200	24,364	41,013	24,213	348,760	0	532,307	479,794	1,009,251
13	33	4	275	300	160,816	630,364	64,817	1,917,598	181,164	2,043,022	1,933,344	2,722,991
14	46	5	188	200	174,456	469,310	60,926	884,966	150,361	744,540	691,714	889,121
15	33	4	275	300	169,219	704,659	68,310	1,939,105	14,972	1,837,597	1,518,458	2,300,440
16	19	5	188	400	110,897	639,653	54,674	2,268,795	35,365	2,636,373	1,450,628	1,814,069
17	19	5	363	200	20,208	49,798	25,373	389,627	0	813,615	824,552	1,369,793
18	19	2	188	400	123,741	785,580	60,990	2,961,491	12,110	2,340,317	863,572	1,437,536
19	33	4	275	300	181,066	781,895	79,243	2,010,017	19,449	1,915,652	1,866,710	3,017,536
20	46	5	188	400	174,724	732,236	82,324	2,243,006	125,893	1,959,065	1,530,885	2,302,503
21	46	2	188	200	192,742	477,141	76,445	955,343	2213	474,777	411,619	549,926
22	19	2	188	200	159,844	431,398	57,229	11,480,766	588	520,127	556,393	736,702
23	46	5	363	400	39,785	79,412	43,339	394,592	21,319	860,004	952,253	1,643,192
24	46	5	363	200	24,928	38,607	23,739	244,447	2444	514,785	601,826	1,196,517
25	46	2	188	400	244,094	1,130,428	112,012	3,664,747	16,320	2,050,746	1,457,251	2,536,035
26	19	5	188	200	164,738	561,013	65,663	1,156,150	7126	1,264,879	1,042,417	1,582,602
27	19	5	363	400	23,549	68,639	24,894	401,562	22,152	1,151,287	982,853	1,248,495
28	46	2	363	400	57,628	84,163	52,175	568,516	31,196	734,918	755,387	1,155,230
29	33	4	275	300	150,996	657,507	64,570	1,801,181	33,295	1,885,199	1,625,653	2,480,537
30	19	2	363	400	29,845	73,125	31,792	526,487	36,768	943,367	582,297	851,607

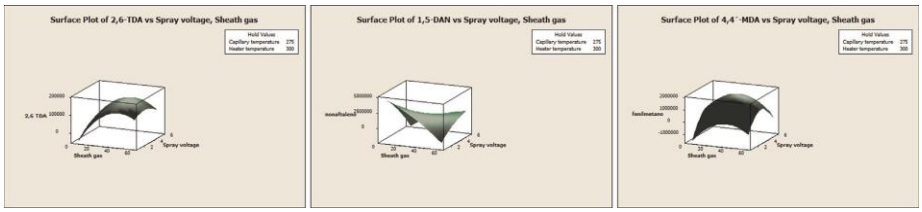


Fig. 1. Three-dimensional response surface for 2,6-TDA, 1,5-DAN and 4,4′-MDA.

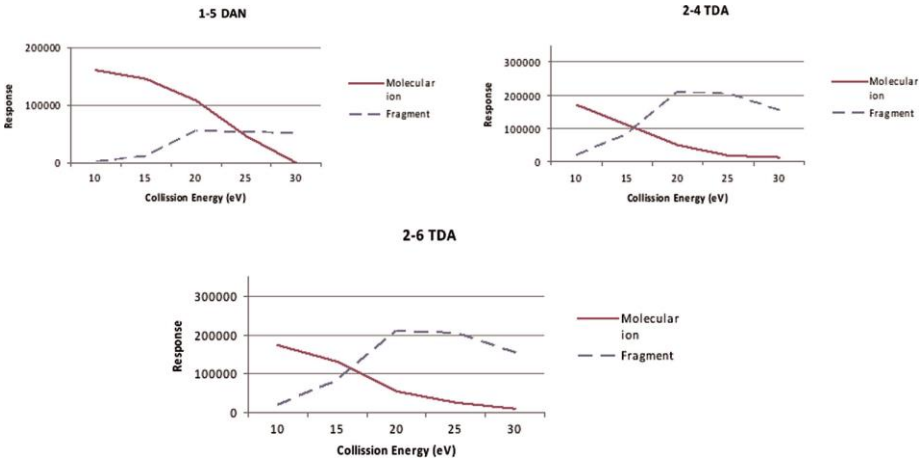


Fig. 2. Responses for molecular ion and fragments of 1,5-DAN, 2,4-TDA and 2,6-TDA at collision energies between 10 and 30 eV in HCD fragmentation.

Table 4
Results (μg/kg) of target compounds of monitorized samples.

Analites	Samples									
	N=1	*N*=2	*N*=3	*N*=4	*N*=5	*N*=6	*N*=7	*N*=8	*N*=9	*N*=10
1,5-DAN	–	–	–	–	–	–	–	–	–	–
2,4-TDA	9.04	12.04	–	–	–	–	–	–	–	–
2,6-TDA	2.67	4.7	–	–	–	–	–	–	–	–
4,4′-MDA	10,446	19,715	–	2.4	–	2.7	2.5	–	–	2
3,3′-DMB	49	–	–	–	–	–	–	–	2.7	–
4,4′-DPE	–	–	–	–	–	–	–	–	–	–
m-PDA	–	2	–	–	–	–	–	–	–	–
Aniline	284	283	–	–	3.5	–	2.6	2.5	–	–

(–)= <LQ.

effect must be evaluated and discussed in the context of method development, and appropriate calibration technique, compensating for these effects, should be used, if necessary.

ME was studied as described by Matuszewsky et al. [24]. Thus, two different sets of solutions were prepared (set A: standard solutions in mobile phase; set B: spiked blank of simulant) and determined using the ESI interface and the optimized factor setting. The absence or presence of matrix effects on the quantification was evaluated by comparing the absolute peak areas of the two sets (ME%=B/A × 100). Both A and B sets had concentrations of 15 ng mL^{-1}. The matrix effect was classified into three different categories attending to the calculated values. There was no matrix effect when the ME factor was between 80% and 120%, because the repeatability of the results would be close to this range. A medium matrix effect was considered when the values ranged between 40% and 80% or 120% and 150%. A percentage below 40% or above 150% was classified as a high matrix effect. The majority of amines showed ion suppression. 4,4′-DPE, 2,4-TDA, 2,6-TDA and 1,5-DAN presented moderated matrix effect with values of ME ranging from 60% to 80%. Others such as aniline and m-PDA had high ion suppression, ranging from 20% to 50%. For 4,4′-MDA and 3,3′-DMB no matrix effect was observed.

A compensation approach, such as the use of matrix-matched standards, is considered to be a useful method for eliminating the

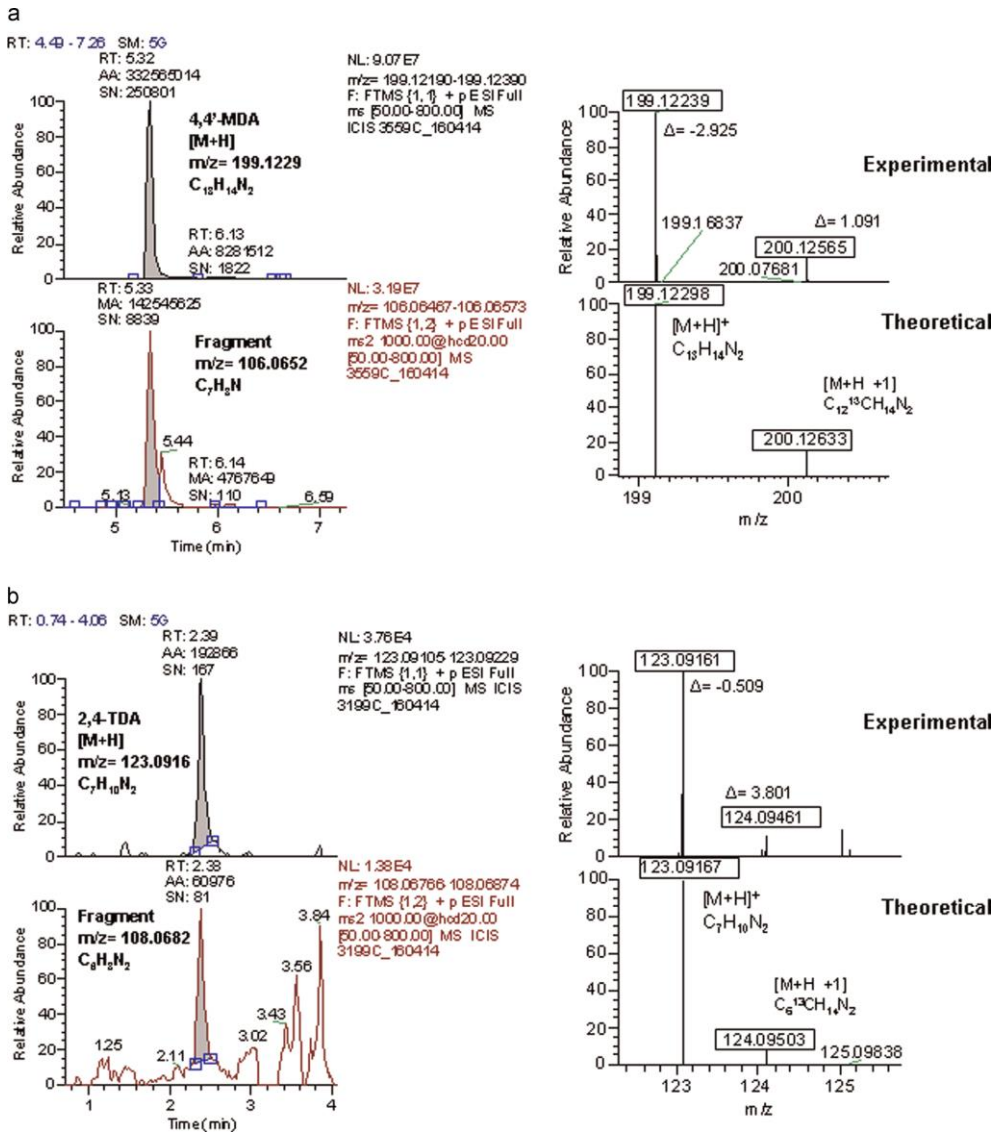


Fig. 3. Extracted ion chromatograms of the parent ions ([M+H]) and selected fragments (left), along with experimental and theoretical isotopic pattern (right) for (a) 4,4′-MDA and (b) 2,4-TDA in real samples.

consequences of matrix effects on the reliability (accuracy and precision) of the data. Consequently, a matrix-matched calibration curve was used for quantification.

A complete validation was carried out for all target compounds. Matrix-matched calibration plots showed good linearity between 1.5 or 2 and 20 ng mL^{-1} in vial. The specificity of the method was tested by analysis of blank matrixes. The absence of any chromatographic peak at exactly the same retention time of the target compound indicated there were no matrix compounds that might give a false positive signal in these samples. Table 2 reports other validation

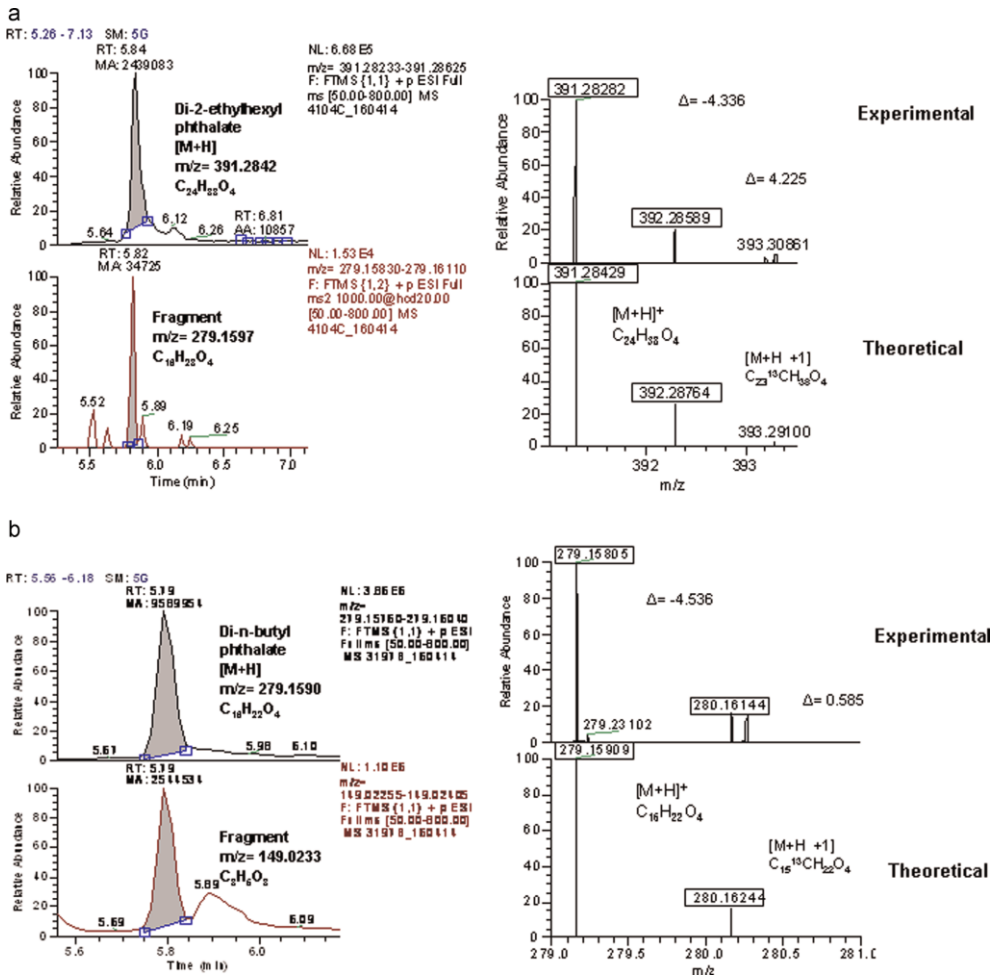


Fig. 4. Post-target screening analysis: extracted ion chromatograms of the parent ions ([M+H]) and selected fragments (left), along with experimental and theoretical isotopic pattern (right) for (a) di-2-ethylhexyl phthalate and (b) di-n-butyl phthalate in real samples.

results including recovery (accuracy), coefficient of variation (precision) and limits of quantification (LOQ). Mean recoveries ranged from 78% to 121%, with coefficients of variation below 15%. The LOQ of the whole method was 2.5 μg/kg^{-1} for ANL and 3.3′-DMB, and 2 μg/kg^{-1} for the rest of the primary amines. These LOQs were low enough to be used in the official monitoring of PAAs from kitchen utensils according to EU regulation 10/2011 [3].

A similar sensitivity was reported by Mortensen et al. [4] (the authors only presented limits of detection) in a method for 20 PAAs using LC–MS/MS in aqueous food simulants for the four common PAAs (m-PDA; 2,4-TDA, 4,4′-MDA; 3,3′-DMB). However, more recently Mattarozzi et al. [9] have developed a method for PAAs using LC-HRMS, achieving lower LOQ for ANL, 2,4-TDA and 4,4′-MDA. Nevertheless, the method was applied to a different matrix (plastic laminate materials) and a different approach for LOQ calculation was used. This method was validated for a wider scope of substances (22 PAAs) but did not include a post-target approach.

3.5. *Target and post-target analysis of real samples*

Both quantitative target analysis and post-target screening analysis were applied to 10 real kitchen utensils. Six out of eight target PAAs were detected in at least one sample (see Table 4), of which 4,4′-MDA and ANL were detected in six and five samples, respectively. In two black-nylon utensil samples concentrations were found to be higher than the regulatory limits for 4,4′-MDA (10,446 and 19,715 μg kg^{-1}) and for ANL (283 and 284 μg kg^{-1}) were found. As an example of identity confirmation of the detected target analytes,

Fig. 3 shows the accurate-mass extracted chromatograms of the parent ion and the characteristic fragments obtained for 4,4′-MDA and 2,4-TDA, with a mass window of 10 ppm (5 ppm mass error tolerance). The theoretical and experimental isotopic patterns (12C/13C) of these amines are also shown. In both cases, the established identification criteria are met.

These results are in agreement with other studies. For example, Sendón et al. [6] also found 4,4′-MDA and ANL as the main compounds in cooking utensils, with 4,4′-MDA presenting higher concentrations. This compound is used in the manufacture of some types of polyamide to increase the stability of the plastic at high temperatures. It has also been suggested that 4,4′-MDA is used to produce the azodyes that give the utensils their black colour. In some samples, 2,4-TDA and 3,3′-DMB were also identified, but in lower concentrations.

Mortensen et al. [4] also observed that the predominant PAA migrant from black nylon cooking utensils were 4,4′-MDA and aniline. It was suggested that the source of PAAs was the application of black colourant in the polyamide raw material. In addition, Trier et al. [8] collected 22 black nylon kitchen utensils from Danish importers. They found that the majority of samples with violations came from China (migration of both 4,4′-MDA and ANL), with Sweden (migration of ANL) being the second largest contributor.

Automated and fast data processing is required for comprehensive screening of large database compounds. The main advantage of this approach is that a comprehensive search of a large number of compounds can be performed, without the need for standards. Using the created database (Table SI-1), the software automatically flags the compounds that meet the screening identification criteria. These criteria should, at the same time, allow having virtually no false negatives and provide an acceptable number of false positives. The reduction of false negatives could be improved including additional diagnostic ions such as adducts, isotope ions and fragments [22].

Two out of the 70 compounds included in the database were identified in the 10 nylon kitchen utensils analysed. Fig. 4 shows the accurate mass extracted chromatograms of the parent ion and the characteristic fragments for di-n-butylphthalate and di-2-ethylhexyl phthalate, with the theoretical and experimental isotopic pattern in two positive samples. For a final confirmation, standards of the identified substances were injected. Di-n-butylphthalate was confirmed in all samples and the di-2-ethylhexyl phthalate only in two of them. Both compounds are commonly used plasticizers or adhesives due to their suitable properties and their low cost [25–27].

The post-target screening approach permits the investigation of new compounds added to the database by only reprocessing the full-scan accurate-mass raw data in a retrospective analysis [28].

4. Conclusions

The analytical capabilities of the platform integrated by liquid chromatography coupled to single-stage orbitrap high resolution spectrometer have been evaluated for the implementation of target analysis of regulated PAAs and post-target screening analysis of 77 food-contact migrants in nylon kitchen utensils. For the quantitative target analysis of 8 PAAs, the developed method presents appropriate performance parameters, suitable for official enforcement control. The primary aromatic amines more frequently detected in kitchen utensils were 4,4′-methylenedianiline and aniline, with concentrations ranging from 2.4 to 19,715 $\mu g\ kg^{-1}$ and 2.5 to 283 $\mu g\ kg^{-1}$, respectively.

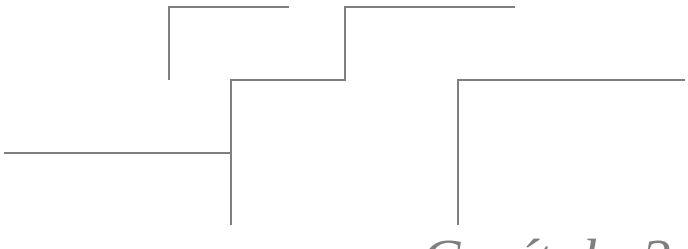
The post-target screening analysis using a customized theoretical database permits the tentative identification of migrating substances present in the food simulant without the necessity of reference standards or additional analysis. Di-n-butylphthalate and di-2-ethylhexyl phthalate substances, which were not initially included in the target analysis, have been identified. These compounds were finally confirmed with injected standards.

Appendix A. Supplementary material

Supplementary material associated with this article can be found in the online version at 10.1016/j.talanta.2015.03.026.

References

[1] C. Brede, I. Skjervak, H. Herikstad, J. Chromatogr. A 983 (2003) 35–42.
[2] International Agency for Research on Cancer (IARC), Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Available online at: ⟨http://monographs.iarc.fr/ENG/Classification/index.php⟩.
[3] European Commission, Off. J. Eur. Union L12 (2011) 1.
[4] S.K. Mortensen, X.T. Traer, A. Forverskov, J.H. Petersen, J. Chromatogr. A 1091 (2005) 40–50.
[5] Rapid Alert System for Food and Feed (EU) Annual Report 2008. Available online at: ⟨http://ec.europa.eu/food/safety/rasff/docs/rasff_annual_report_2008_en.pdf⟩.
[6] R. Sendón, J. Bustos, J. Sanchez, P. Paseiro, M.E. Cirugeda, Food Addit. Contam. Part A 27 (2010) 107–117.
[7] D. Pezo, M. Fedeli, O. Bossetti, C. Nerón, Anal. Chim. Acta 756 (2012) 49–59.
[8] X. Trier, B. Okholm, A. Foverskov, M.-L. Binderup, Food Addit. Contam. 27 (2010) 1325–1335.
[9] M. Mattarozzi, Francesca Lambertini, Michel Suman, Maria Careri, J. Chromatogr. A 1320 (2013) 96–102.
[10] C. Simoneau, E. Hoekstra, E. Bradley, J. Bustos, V. Golja, O. Kappenstein, D. Kalsbeek, J. Keegan, M.R. Milana, K. Cwiek-Ludwicka. J. Petersen, M. Polz, P. Sauvegran, F. Vanhee, EN 24815 EN 2011, JRC Scientific and Technical Reports, 1st edition, 2011.
[11] J.F. Garcia-Reyes, M.D. Hernando, A. Molina-Diaz, A.R. Fernandez-Alba, Trends Anal. Chem. 26 (2007) 828–841.
[12] A. Kaufmann, Anal. Bioanal. Chem. 403 (2012) 1233–1249.
[13] R.J.B. Peters, A.A.M. Stolker, J.G.J. Mol, A. Lommen, E. Lyris, Y. Angelis, A. Vonaparti, M. Stamou, C. Georgakopoulos, M.W.F. Nielen, Trends Anal. Chem. 29 (2010) (2010) 1250–1268.
[14] Technical Guidelines on Testing the Migration of Primary Aromatic Amines from Polyamide Kitchenware and of Formaldehyde from Melamine Kitchenware (EUR24815EN) (first ed.) 2011.
[15] European Commission, Guidance document (SANCO) No. 12571/2013 of 01 January 2014 on Analytical Quality Control and Validation Procedures for Pesticides Residues Analysis in Food and Feed. ⟨http://www.eurl-pesticides.eu/⟩.
[16] Eurachem Guide, The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics, LGC, Teddington Ltd., 2014. ⟨http://www.eurachem.org/⟩ (first English ed.1.0).
[17] William P. Gardiner, Bill Gardiner, Statistical Analysis Methods for Chemists, Elsevier, Amsterdam, 1997.
[18] D.L. Massart, B.G.M. Vandeginste, C.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smellers, V.R. Beke, Handbook of Chemometrics and Qualimetrics, Elsevier, Amsterdam, 1997.
[19] K. Danzer, Ll. Currie, Guidelines for Calibration in Analytical Chemistry, IUPAC, Great Britain, 1998.
[20] H. Gallart-Ayala, O. Nuñez, P. Lucci, Trends Anal. Chem. 42 (2013) 99–124.
[21] M.L. Gómez-Pérez, R. Romero-González, P. Plaza-Bolaños, E. Génin, J.L. Martínez Vidal, A. Garrido Frenich, J. Mass Spectrom. 49 (2014) 27–36.
[22] H.G.J. Mol, P. Zomer, M. de Koning, Anal. Bioanal. Chem. 403 (2012) 2891–2908.
[23] C. Coscollà, V. Yusà, P. Martí, A. Pastor, J. Chromatogr. A 1200 (2008) 100–107.
[24] B.K. Matuszewsky, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019–3030.
[25] Tzung-Hai Yen, Dan-Tzu Lin-Tan, Ja-Liang Lin, J. Formos. Med. Assoc. 110 (2011) 671–684.
[26] U. Heudorf, V. Mersch-Sundermann, J. Angerer, Int. J. Hyg. Environ. Health 210 (2007) 623–634.
[27] E. Fasano, F. Bono-Blay, T. Cirillo, P. Montuori, S. Lacorte, Food Control 27 (2012) 132–138.
[28] M. Ibáñez, J.V. Sancho, L.S. Bijlsma, A.L.N. van Nuijs, A. Covaci, F. Hernández, Trends Anal. Chem. 57 (2014) 107–117.



Capítulo 2

4.2. Capítulo 2. Análisis de fotoiniciadores y aminas aromáticas primarias en distintos tipos de envases y materiales en contacto con alimentos por UHPLC-HRMS

4.2.1. Resumen

Los fotoiniciadores son compuestos arílicos aromáticos no saturados muy sensibles a la energía radiante. Las moléculas del fotoiniciador se descomponen al recibir energía radiante y forman radicales libres o cationes (Bishop 2011). Por tanto, los fotoiniciadores cumplen la función crítica de iniciar la polimerización por radicales libres o catiónica en los materiales. El fotoiniciador se utiliza en gran medida en los envases; es un tipo de aditivo para la curación o polimerización de la pintura con la luz ultravioleta. Una tinta o recubrimiento que contenga un pequeño porcentaje de fotoiniciador se puede secar en menos de un segundo bajo una radiación de luz UV (Aznar et al. 2015, Gallart-Ayala et al. 2011). Los recubrimientos y tintas se disuelven completamente, y no producen sustancias volátiles durante el proceso de curación. Además, la curación por UV requiere menos energía que la curación tradicional, y el proceso de curado UV muestra resultados mucho mejores de recubrimiento con un acabado brillante y mayor resistencia al rayado de la superficie del revestimiento (Bishop 2011, Gruber 1992).

La presencia de componentes de tinta de impresión en los envases de alimentos se ha convertido en un motivo de preocupación ya que pueden migrar al alimento. Fotoiniciadores como 4- metilbenzofenona o el 2-ITX, han sido motivo de alerta debido a su presencia en alimentos (RASFF 2005, EFSA 2009). El uso de tintas de impresión para el envasado de alimentos no está regulado por las normativas europeas.

Las aminas aromáticas, aparecen en los materiales en contacto con alimentos, como son los envases, films y los utensilios de cocina, fruto de la incorporación del poliuretano como adhesivo en estos materiales, o por la degradación de los grupos azoicos utilizados como colorantes en utensilios de cocina de nylon y otros plásticos (Brede et al. 2003)

Los fotoiniciadores de tinta UV y las aminas aromáticas primarias (PAAs) tienen características fisicoquímicas similares; son compuestos polares con baja volatilidad y estabilidad térmica, por tanto, pueden ser analizados por técnicas analíticas similares. LC-MS ha sido la técnica utilizada para el análisis de fotoiniciadores y PAAs en envases de plástico (Aznar et al. 2015, Vavrouš et al. 2016, Aznar et al. 2012) y utensilios de plástico

(Sanchis et al. 2015) en los últimos años. Gallart-Ayala et al. (2013), revisó los métodos analíticos para los MCA usando cromatografía líquida hasta 2013, concluyendo que MS/MS (QqQ) sigue siendo el método de elección en el análisis de contaminantes de envases de alimentos. Sanchis et al. 2018, incluyó en su trabajo el uso de UHPLC-HRMS, una herramienta analítica que permite el desarrollo de estrategias analíticas combinando (a) análisis de contaminantes dirigido (determinación de analitos prioritarios específicos para los cuales se dispone de estándares y para los cuales la masa exacta , la ventana de tiempo de retención, el patrón isotópico y los fragmentos son las herramientas de identificación) y (b) análisis de cribado retrospectivo o posterior, basado en una base de datos personalizada de moléculas conocidas con inclusión de algunos iones de fragmento de diagnóstico o patrón isotópico.

En este estudio se ha desarrollado una estrategia analítica que combina el análisis *target* (dirigido) cuantitativo para fotoiniciadores y PAAs (18 analitos) con análisis *suspect screening* (retrospectivo) basado en la identificación de los compuestos mediante una base de datos con 87 sustancias utilizando el UHPLC-Orbitrap-HRMS (figura 13).

La extracción de sustancias para los tres materiales estudiados se llevó a cabo de dos maneras diferentes, (1) Migración estandarizada de acuerdo con el Reglamento 10/2011 y (2) Ensayo destructivo consistente en someter a migración todo el envase o MCA.

Migración estandarizada

El ensayo de migración de los materiales estudiados (bolsas, tetrabriks y envases para derivados lácteos) se llevó a cabo siguiendo el Reglamento 10/2011, relativo a materiales plásticos. En este trabajo se sometieron a ensayo bolsas para pulpa de fruta y envases para derivados lácteos. En cuanto a los envases para derivados lácteos y tetrabriks, están compuestos por múltiples capas, de forma que la regulación sólo se aplicaría a la capa interna en contacto con el alimento, fabricada con plástico. La migración en tetrabriks y envases (derivados lácteos) consistió en un ensayo por llenado, ya que, la parte interna de la muestra está destinada a contactar con el alimento. Sin embargo, para las bolsas de pulpa de fruta, la capa destinada al contacto con el alimento se extendió en una celda de acero inoxidable fabricada por Merck y se puso en contacto con el simulante. Las condiciones de temperatura, tiempo de contacto y simulante para cada ensayo fueron los detallados en la tabla 15.

Ensayo destructivo

Se aplicó un método no estandarizado para identificar las sustancias presentes en cada una de las capas tanto de la bolsa para pulpa de fruta como del tetrabrik (envase multicapa). Para ello las bolsas se cortaron en partes de 1cm^2 y luego todas las porciones fueron sumergidas en 100 mL de ácido acético al 3%, durante 2 horas a 70º C. A continuación, se filtró el simulante y se separó de las piezas del material. Sobre 6 mL de simulante (ácido acético al 3%) extraído se adicionaron 2 ml de diclorometano (DCM) (extracción liquido-liquido). El extracto se evaporó a sequedad con una corriente de nitrógeno, y fue reconstituido con 200 µL de metanol-agua.

Tabla 15. Condiciones experimentales de los ensayos de migración

Muestra	Simulante	Temperatura (ºC)	Tiempo (días)
Tetrabrik	Ácido acético 3% (B)	40	10
Tetrabrik	Etanol 50% (D1)	20	10
Bolsas de pulpa	Etanol 50% (D1)	20	10
Envases (derivados lácteos)	Etanol 50% (D1)	40	10

Para la optimización del método analítico cuantitativo (target) se ha estudiado la fragmentación de las sustancias y el efecto matriz. El método se ha validado con objeto de comprobar su utilidad para el control de estas sustancias.

La metodología analítica se ha aplicado a materiales plásticos como tetrabriks (para zumo y leche), bolsas (para pulpa de fruta) y envases (para derivados lácteos) de muestras recogidas en industrias de la Comunitat Valenciana (España). Es el primer método analítico en la literatura, que combina fotoiniciadores y aminas con análisis cuantitativo y estudio retrospectivo en plásticos.

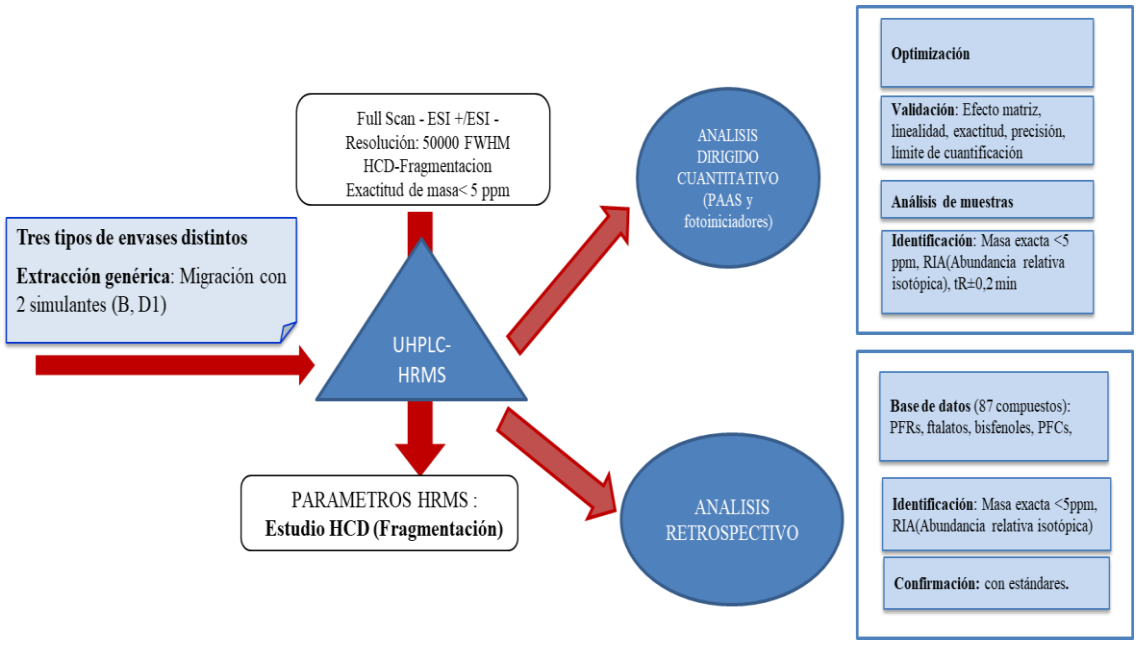


Figura 13. Estrategia de análisis de fotoiniciadores y aminas en distintos envases por UHPLC-HRMS

4.2.2. Discusión de resultados

a. Estudio de fragmentación (HCD)

Para seleccionar la energía de colisión más adecuada y así obtener la mejor respuesta de los fragmentos del compuesto, se trabajó con energía de colisión en el rango de 10 – 30eV. La figura 14 muestra la respuesta obtenida para algunas aminas (ANL) y fotoiniciadores (DEAB y DETX) aplicando diferentes energías de colisión. La respuesta para anilina fue mejor en 30eV, sin embargo, para otros compuestos, la energía de colisión optimizada fue de 20eV. Finalmente, se seleccionó una energía de colisión de compromiso de 20eV. Esta energía de colisión proporcionó buena sensibilidad para la mayoría de los fragmentos.

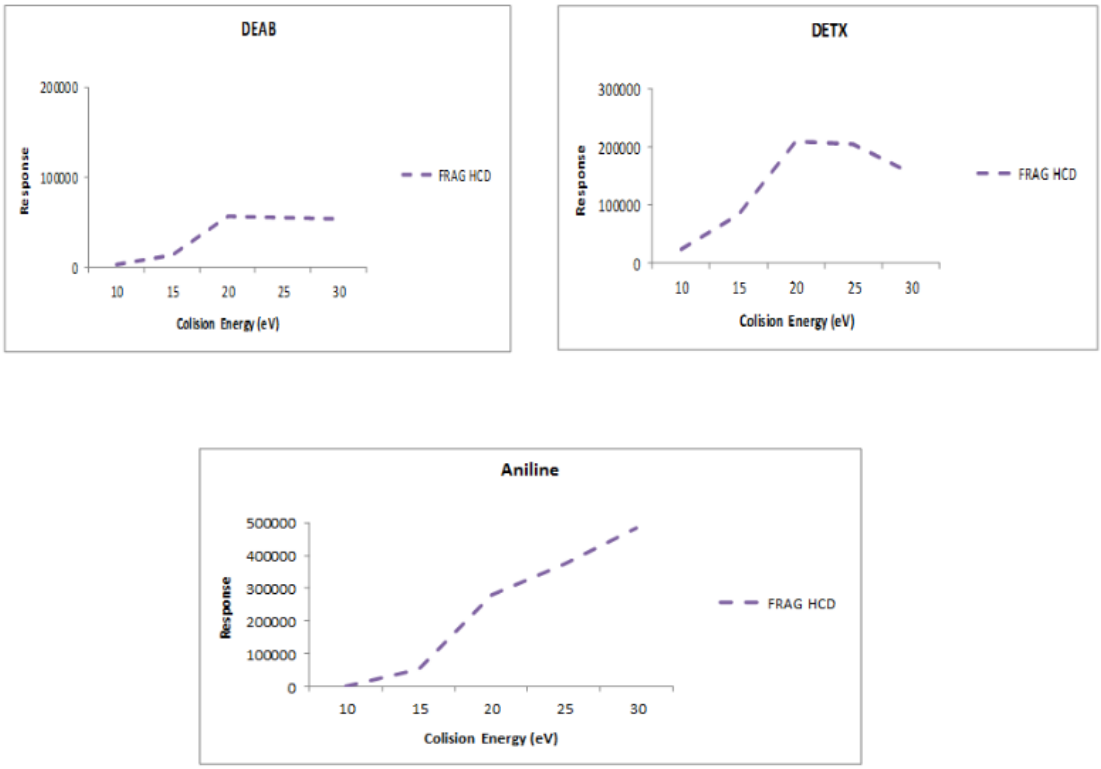


Figura 14. Fragmentación HCD para los fotoiniciadores DEAB y DETX y la Anilina a las diferentes energías de colisión de 10 a 30 Ev

b. Estudio del efecto matriz y validación

La mayoría de las aminas mostraron un efecto matriz de supresión iónica (tabla 16). En el simulante B (ácido acético 3%), 4, 4 ′-DPE, 2, 4-TDA, 2, 6-TDA y 1,5-DAN presentaron un efecto matriz moderado, con valores entre el 60% y el 80%. Otros, como la anilina y la m-PDA, tenían una alta supresión iónica, que oscilaban entre el 20% y el 50%. No se observó ningún efecto de matriz en 4, 4 ′-MDA y 3, 3 ′ DMB. Con respecto a los fotoiniciadores, BP y ITX presentaron un moderado efecto matriz, con valores en el rango de 70% a 90% y para los fotoiniciadores, HMPP, HCPK, EDMAB, DMPA, PBZ, DEAB, DETX y EHDAB no se observó efecto matriz en simulante B.

En el simulante D1 (etanol al 50%), 4, 4 ′-DPE, 4, 4 ′-MDA y 3, 3 ′-DMB no se observó efecto matriz. En el caso de la anilina, 2, 4-TDA, 2, 6-TDA y m-PDA presentaron un efecto de matriz moderado con valores entre el 70% y el 100%. En cuanto a los fotoiniciadores, no se observó ningún efecto matriz en simulante D1.

Debido a la existencia de moderada supresión iónica en la mayoría de los compuestos fue necesario el uso de técnicas que compensen el efecto matriz para la obtención de buena

reproducibilidad y exactitud. En este trabajo se utilizó la curva de calibrado de 5 puntos en matriz para superar este tipo de efecto. En la validación se obtuvieron resultados satisfactorios en este sentido, con coeficientes de determinación en las curvas de calibrado en matriz $R^2 > 0{,}99$ y valores de recuperaciones en el rango 72-120% con precisión menores al 20% en todos los analitos. En la figura 15 se pueden observar los cromatogramas de algunos de los analitos estudiados en una adición al límite de cuantificación.

Tabla 16. Efecto matriz absoluto (MEabs%) en los simulantes B y D1.

Efecto Matriz Absoluto (MEabs%)

Compuestos	**HCPK**	**DMPA**	**DETX**	**EHDAB**	**HMPP**	**ITX**	**DEAB**	**PBZ**	**EDMAB**
B simulante	118	101	86	101	106	75	114	86	101
D1 simulante	91	88	111	84	88	89	111	80	92
Compuestos	**BP**	**1,5 DAN**	**2,4 TDA**	**3,3' DMB**	**2,6 TDA**	**4,4' MDA**	**4,4' DPE**	**ANL**	**M-PDA**
B simulante	84	60	72	97	77	99	71	50	42
D1 simulante	92	64	70	95	77	99	81	70	76

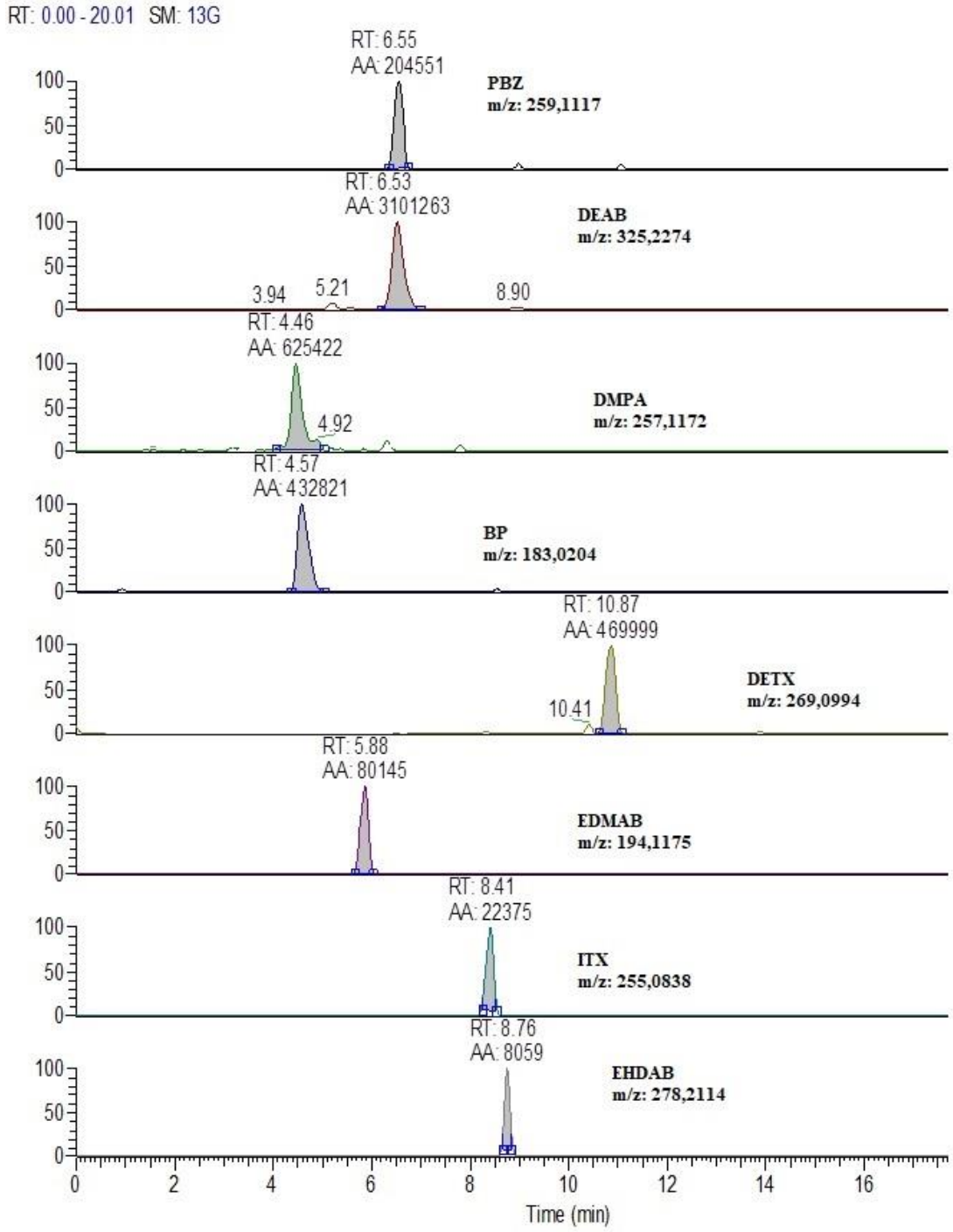


Figura 15. Cromatograma de masa exacta extraídos de varios fotoiniciadores de interés en una muestra de simulante adicionada al LC

c. Aplicación a muestras reales. Análisis dirigido (target) y análisis retrospectivo

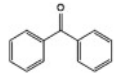
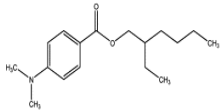
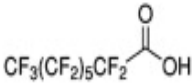
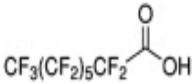
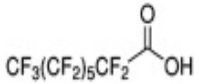
(1) Migración estandarizada

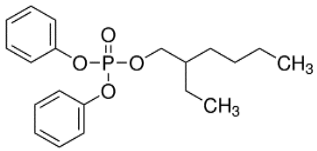
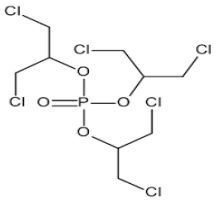
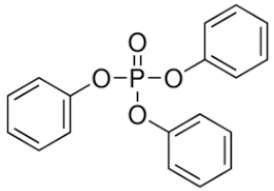
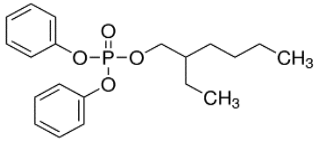
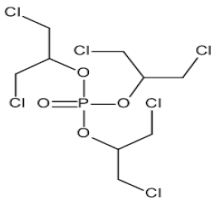
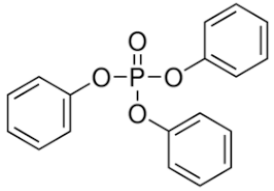
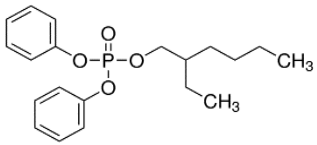
Se analizaron seis bolsas para pulpa de fruta, seis tetrabriks y seis envases para derivados lácteos con el método estandarizado, según el Reglamento 10/2011. No se encontró ningún compuesto en los simulantes migrados. La ausencia de compuestos como fotoiniciadores, compuestos fluorados presentes en plásticos o cartones podría confirmar que la aplicación de una capa de aluminio en tetrabriks y bolsas puede reducir o incluso eliminar la migración de estos compuestos en alimentos o simulantes.

(2) Ensayo destructivo

Se realizaron ensayos de migración con ambos simulantes (B y D1) para los materiales multicapa en las tres matrices. El término multicapa comprende: la parte exterior sin alimentos (lado exterior), el lado de contacto con alimentos (lado interno) y las capas de barrera (aluminio). La tabla 17 muestra los compuestos detectados en muestras reales por el ensayo destructivo, tanto en el modo dirigido cuantitativo (*target*) como en el modo retrospectivo (*suspect screening*).

Tabla 17. Compuestos detectados en muestras reales por el test destructivo

	Análisis target			**Retrospectivo**		
Compuestos	**Tetrabriks**	**Bolsas**	**Envases**	**Tetrabriks**	**Bolsas**	**Envases**
Fotoiniciadores	**BP** 	**EHDAB** 	-	-	-	-
PAAs	-	-	-	-	-	-
PFCs	-	-	-	**PFOA**  **PFOS** CF_3-$(CF_2)_7$-SO_3H	**PFOA**  **PFOS** CF_3-$(CF_2)_7$-SO_3H	**PFOA**  **PFOS** CF_3-$(CF_2)_7$-SO_3H

PFRs	-	-	-	**EHDPP**  **TCPP**  **TPhP** 	**EHDPP**  **TCPP**  **TPhP** 	**EHDPP** 

En la figura 16 se muestran los cromatogramas de dos muestras en las que se detectó Benzofenona en tetrabriks y EHDAB en bolsas para pulpa de fruta en ambos simulantes (B y D1) mediante el uso del método target.

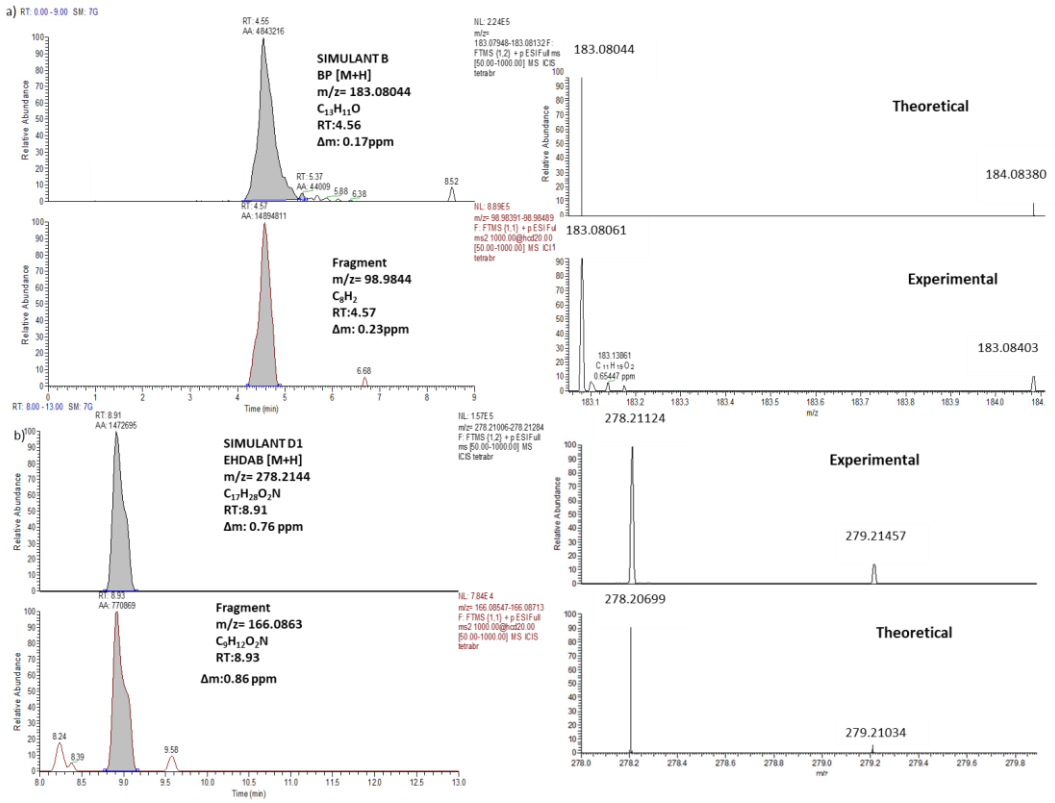


Figura 16. Cromatograma de masa extraída del ion diagnóstico y su confirmación de (a) BP y (b) EHDAB en dos muestras reales utilizando el análisis target (cualitativo)

Por otro lado, se realizó el análisis retrospectivo de las muestras mediante comparación con la base de datos creada. En este análisis se identificaron tentativamente varias sustancias, de las que únicamente se podrá realizar la identificación inequívoca mediante la adquisición del estándar correspondiente o mediante otras técnicas de espectrometrías de elucidación estructural. Este cribado dio como resultado el hallazgo de dos compuestos perfluorados (PFCs) mostrados en la tabla 17, PFOA y PFOS, identificados en tetrabriks, envases para derivados lácteos y bolsas para pulpa de fruta. La figura 17 muestra el cromatograma de los iones diagnóstico de PFOA en envases para derivados lácteos.

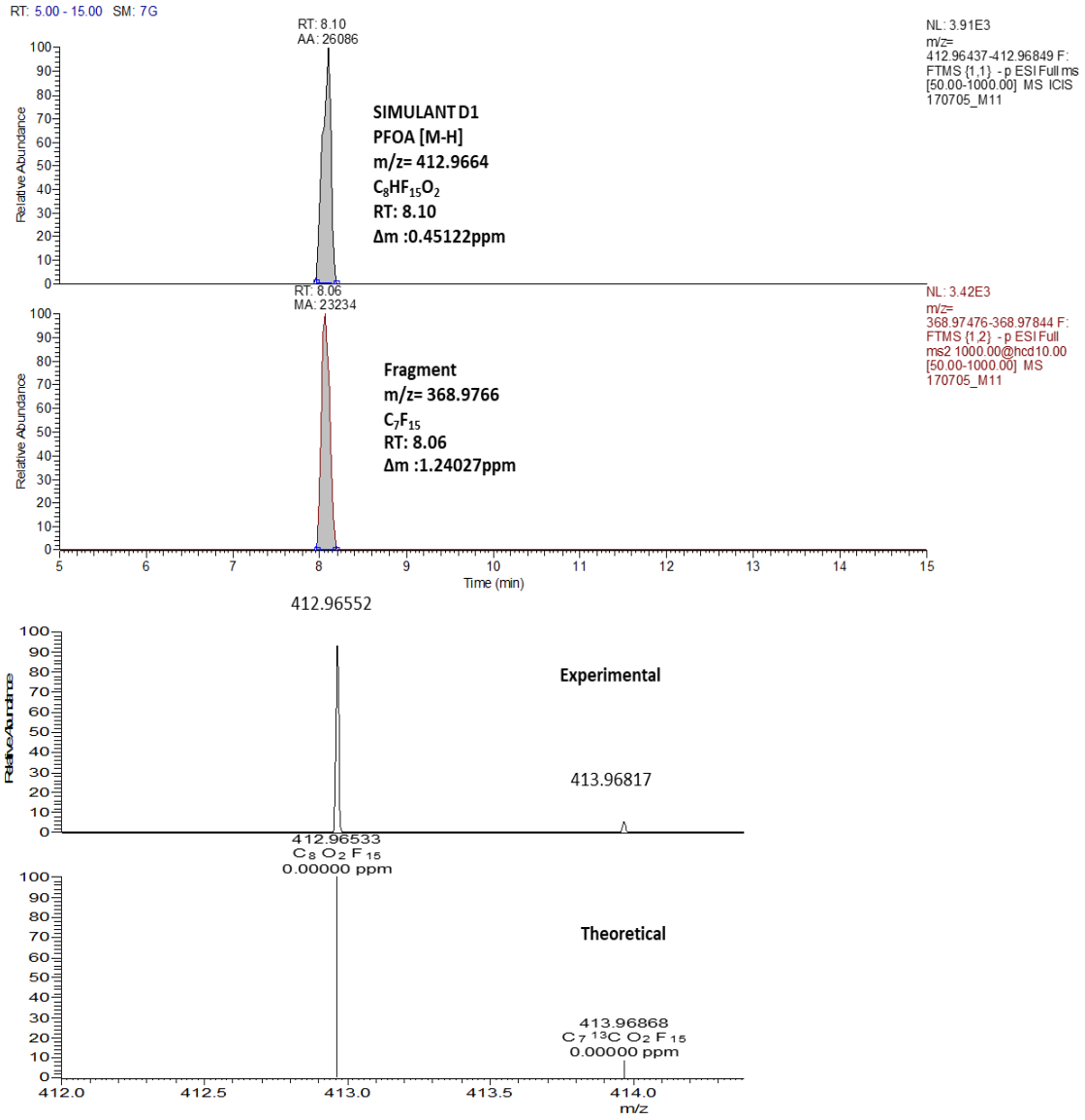


Figura 17. Cromatograma de masa extraída del ion diagnóstico y su confirmación de PFOA en muestra real mediante el análisis *postarget* (cualitativo)

Además, tres retardantes de llama ó PFRS, EHDPP, TCCP y TPHP fueron identificados en el análisis *postarget* en tetrabriks y envases destinado a derivados lácteos. La figura 18 presenta el cromatograma de los iones diagnostico para EHDPP en una muestra real de tetrabrik. Un PFRS (EHDPP) fue detectado en las bolsas destinadas a contener pulpa de fruta (solución migrada de simulante).

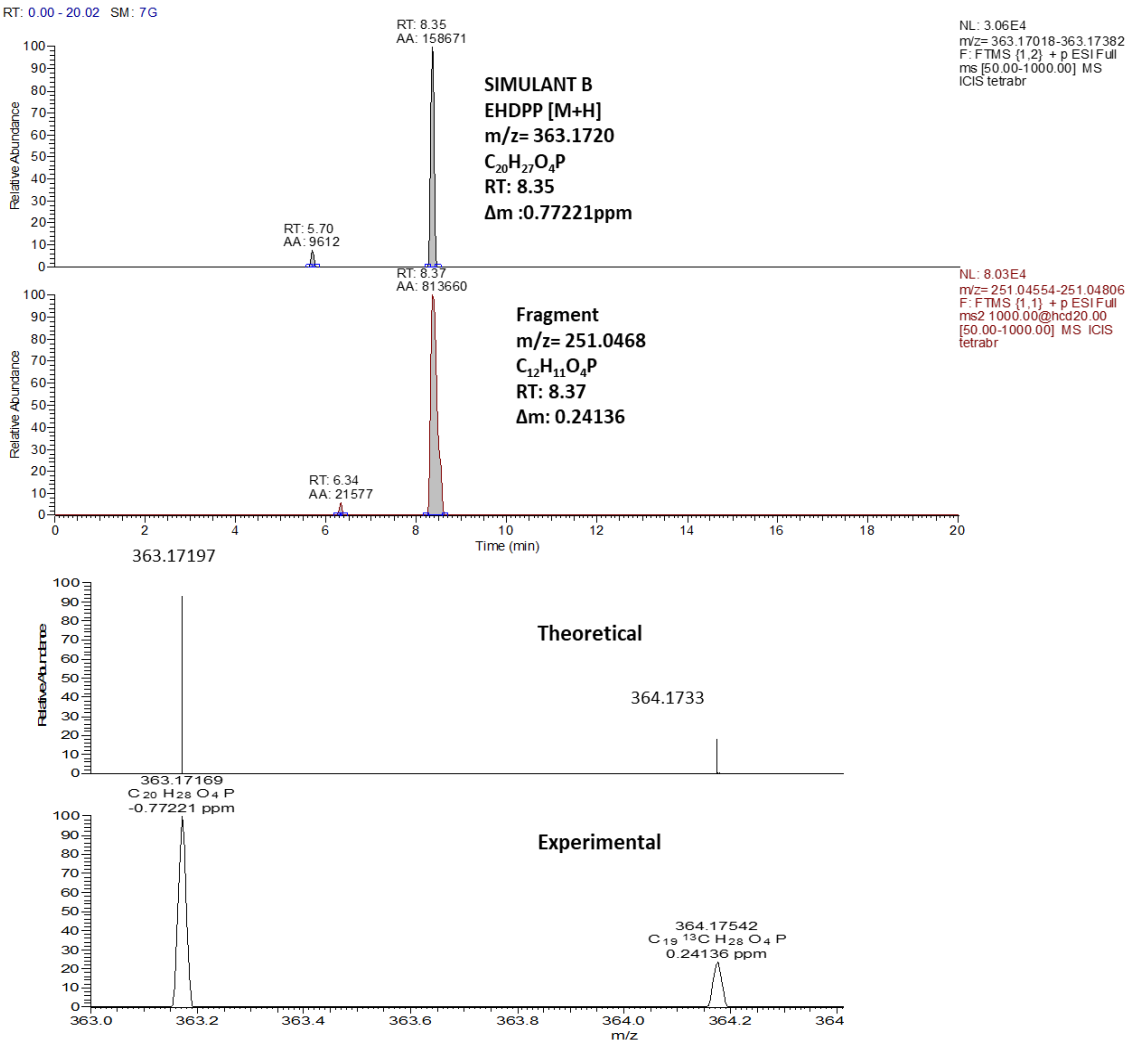


Figura 18. Cromatograma de masa extraída del ion diagnóstico y su confirmación de EHDPP en muestra real mediante el análisis *postarget* (cualitativo)

4.2.3. Conclusiones

- En el presente trabajo se ha desarrollado una estrategia analítica basada en UHPLC-HRMS que permite un análisis amplio (*target y postarget* o "suspect screening") de fotoiniciadores y aminas en distintos envases para alimentos, y que puede utilizarse en la monitorización de estas sustancias.
- El método cuantitativo se ha desarrollado para 10 fotoiniciadores y 8 aminas, presentando un límite de cuantificación entre 0.5-5 μgkg^{-1} para los fotoiniciadores y 2-2.5 μgkg^{-1} para las aminas con recuperaciones entre el 72 y el 120% con precisiones menores del 20 % en dos tipos de simulantes (B y D1).

- Además de la masa exacta y del perfil isotópico, la identificación de las sustancias *target* y *postarget* (análisis retrospectivo) mejora utilizando los fragmentos generados en la celda de colisión (HCD) del Orbitrap. Sin embargo, es necesaria una optimización previa de la energía de colisión.
- El *postarget* análisis realizado sobre muestras reales mediante comparación con una base de datos de 87 sustancias posibilitó la identificación tentativa de nuevos compuestos presentes en los materiales como perfluorados y retardantes de llama no incluidos inicialmente en el análisis dirigido.

4.2.4. Artículo 3. Comprehensive analysis of photoinitiators and primary aromatic amines in food contact materials using liquid chromatography High-Resolution Mass Spectrometry

Talanta 191 (2019) 109–118

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Comprehensive analysis of photoinitiators and primary aromatic amines in food contact materials using liquid chromatography High-Resolution Mass Spectrometry

Yovana Sanchis[a], Clara Coscollà[a], Vicent Yusà[a,b,*]

[a] Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020 Valencia, Spain
[b] Analytical Chemistry Department, Universit of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain

ARTICLE INFO

Keywords:
High-Resolution Mass Spectrometry
Food packaging
Photoinitiators
Orbitrap
Primary aromatic amines

ABSTRACT

A comprehensive strategy for the analysis of UV-ink photoinitiators and primary aromatic amines (PAAS) in food-packaging materials such as, juice tetrabricks, pouches and bags has been developed using liquid chromatography coupled to Orbitrap High-Resolution Mass Spectrometry (LC-Orbitrap-HRMS). The methodology includes both quantitative target analysis and post-run target screening analysis. The quantitative method was validated after a previous optimisation of the single-stage Orbitrap fragmentation through the Higher-Energy Collisional Dissociation (HCD) Cell. Overall, the quantitative method presented recoveries ranging from 78% to 119%, with a precision (RSD) lower than 20%, for the 18 substances in the scope of the target method. Limit of quantification (LOQ) for UV-inks photoinitiators ranged from 0.5 $\mu g/kg^{-1}$ for Isopropyl Thioxanthone (ITX) and 2-Ethylhexyl 4-(dimethylamino) benzoate (EHDAB) to 5 $\mu g/kg^{-1}$ for the rest of photoinitiators. LOQ for PAAs were 2 $\mu g/kg^{-1}$ except for aniline (ANL) and 3,3′ dimethylbenzidine (3,3′-DMB) which was 2.5 $\mu g/kg^{-1}$ in the two studied simulants (acetic acid 3% and ethanol 50%). For post-run target screening a customized theoretical database, that included Bisphenols, Polyfluorinated compounds (PFCs), Phosphorus Flame Retardants (PFRs) and other substances was built. For identification purposes, a mass accuracy lower than 5 ppm, and some diagnostic ions including isotopes and/or fragments were used.

The strategy was applied to 18 samples collected in the Valencian region (Spain). No compounds were detected when the standardised migration test was applied. However, in the destructive test, benzophenone and EHDAB were determined from tetrabrick and pouch materials. In the post-run target analysis two PFCs (Perfluorooctanoic acid and Perfluoro-1-butanesulfonate) and four PFRs (2-ethylhexyl diphenyl phosphate, tris (2-choloroisopropyl) phosphate, triphenyl phosphate and 2-ethylhexyl diphenyl phosphate) were identified.

1. Introduction

There are hundreds of compounds that can migrate from food contact materials (FCMs). Some of them such as UV-ink photoinitiators and primary aromatic amines (PAAs) have similar physico-chemical characteristics and are prone to be analysed by similar analytical techniques.

UV-ink photoinitiators are commonly used in food-packaging materials and therefore, migration of ink components to food must be studied. Inks, defined as a coloured fluid or paste used for writing, drawing or printing, are mainly composed of a pigment or dye, suspended or dissolved in a solvent. Different groups of materials can be used in the manufacture of food packaging inks such as additives, colorants (pigments, dyes), pigment additives, polymeric resins, solvents or photoinitiators [1]. The presence of printing-ink components coming from food packaging has become a subject of concern since, in September 2005, the Rapid Alert System for Food and Feed (RASFF) published an alert from Italian authorities due to the detection of 2-ITX, a photoinitiator present in UV-cured inks, in baby milk [2]. The use of printing inks for food packaging is not regulated by European Regulations, and only exist some National Regulations guidelines.

The PAAs can migrate to foods from plastics such as kitchen utensils [3] or plastic laminates [4]. These amines are formed by hydrolysis of aromatic isocyanates in polyurethane adhesives [5]and by degradation of azodyes used as colorants in nylon kitchen utensils and other plastic materials. Brede et al. [6] identified the polyamide cooking utensils as a common source of PAAs. Some of the more relevant PAAs are: Aniline

* Corresponding author at: Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020 Valencia, Spain.
E-mail address: yusa_vic@gva.es (V. Yusà).

https://doi.org/10.1016/j.talanta.2018.08.047
Received 9 May 2018; Received in revised form 14 August 2018; Accepted 17 August 2018
Available online 18 August 2018

(ANL), 4–4′ methylenedianiline (4,4′ MDA) and 4–4′ Oxidianilina (4,4′DPE) [3,4]. Regulation (EU) No. 10/2011[7] establishes that the specific migration limit (SML) for the sum of all the PAAs is 10 μg/kg.

The analysis of FCM contaminants can be performed in food, in food simulants or in food-contact materials. Migration on plastics intended to come into contact with food is regulated by Regulation (EU) No. 10/2011 [7]. The selection of simulants, such as ethanol and acetic acid, used in the present work are listed in the same regulation [7]. UV-ink photoinitiators and PAAs are polar compounds with low volatility and low thermal stability. LC–MS has been the selected technique in the analysis of photoinitiators and PAAs in plastic packaging [2,8–14] and plastic utensils [3,15] in the last years. Gallart-Ayala et al. reviewed [16] the analytical methods for FCMs using liquid chromatography until 2013. They concluded that MS/MS (QqQ) continues to be the method of choice in the analysis of food-packaging contaminants. Sanchis et al. included in their work LC-HRMS, a powerful analytical tool that allows the development of analytical strategies combining (i) target analysis (determination of specific priority analytes for which standards are available and for which accurate mass, retention time window, isotopic pattern and fragments are reliable identification tools); (ii) post-run target or retrospective screening analysis based on an accurate-mass customized database of known parent molecules and some diagnostic fragment ions or isotopic pattern, and (iii) non-target with no selection of analytes to be searched [9,17,18].

The LC-HRMS methodology using TOF or Orbitrap mass analysers has been recently introduced for the analysis of PAAs [3], inks [2], PFCs [19] additives [20] and phthalates [21] in FCMs. This technique has allowed the identification, in a non-target analysis, of Non Intentionally Added Substances (NIAS), unknown molecules possibly derived from polycarbonate degradation [19] and phthalates possibly derived from nylon kitchen utensils [3]. Mattarozzi et al. [4] developed a target methodology for the analysis of 22 PAAs from plastic laminates by LC–HRMS, with the LOQ ranging between 0.099 and 5.45 μg/kg [4]. LC–HRMS has also been used for the determination of PAAs from nylon kitchen utensils [3], achieving an LOQ of 2.5 μg/kg.

In some fields such as pesticide and veterinary drug analysis, this technique has increasingly become more popular [22,23] owing to its capacity of using the full-scan acquisition mode with high sensitivity, combined with high resolving power (> 50,000 FWHM) and accurate mass measurements (1–5 ppm).

In a previous published paper, in target analysis we have carried out an analytical method only for PAAs determination in nylon kitchen utensils [3]. In the present study we have developed a comprehensive analytical strategy including new important FCM contaminants in target analysis. These relevant compounds are the photoinitiators substances which are applied as an inks in commonly used food contact material matrices by adults and children. Our study is the first one which has developed and validated photoinitiators in food simulants by LC-HRMS.

Consequently, in this study we developed an analytical strategy that combines quantitative target analysis for UV-ink photoinitiators and PAAs with post-run target screening analysis (identification) based on a comprehensive customized database using LC-Orbitrap-HRMS. The analytical methodology was applied to plastic materials such as juice tetrabricks,pouches and bags samples collected in markets of the Valencian Region (Spain). To our knowledge, no work has previously reported analytical methods combining target/post-target analysis for photoinitiators and PAAs in the aforementioned plastic materials by liquid chromatography-high resolution mass spectrometry.

2. Experimental

2.1. Chemicals and reagents

For the target analysis, high-purity standard photoinitiators [1-hydroxycyclohexyl phenyl ketone 99% (HCPK), 2,2-dimethoxy-2-phenylacetophenone 98% (DMPA), 2,4-Diethyl-9H-thioxanthen-9-one 98% (DETX), 2-Ethylhexyl 4-dimethylamino-benzoate 98% (EHDAB), 2-Hydroxy-2-methylpropiophenone 99% (HMPP), Isopropyl Thioxanthone 99% (ITX), 4,4′-Bis-dimethylamino-benzophenone 98% (DEAB), 4-BenzoyIbiphenyl 98% (PBZ) and benzophenone 99% (BP)] and amines [2,6 Toluendiamine 98% (2,6 TDA), 2,4 Toluendiamine 98% (2,4 TDA), Aniline 98% (ANL), 1,5 Naphthalenediamine 99% (1,5 DAN), 1,3 Phenylenediamine 99% (m-PDA,) 4,4′diaminonaphenylether 98% (4,4′DPE) and 3,3′ dimethylbenzidine 98% (3,3′ DMB)] were supplied by Sigma Aldrich.

Individual stock standards were prepared by dissolving 25 mg of pure standard in methanol. Mix working solutions at 5 μg mL^{-1} were prepared with methanol. Two solutions at 100 ng mL^{-1} were prepared with acetic acid (AcH, 3%) and ethanol 50%, respectively. Calibration solutions from 0.4 to 160 ng mL^{-1} were prepared by adding variable volumes of the mix working solutions into two simulants (3% AcH in water, w/v) and (50% ethanol in water, w/v)

Regarding the post-run target analysis, all commercial standards of Phosphorus Flame Retardants (PFRs) 2-ethylhexyl diphenyl phosphate (EHDPP), tris(2-choloroisopropyl) phosphate (TCPP) and triphenyl phosphate (TPhP) were of high purity (> 95%) and were supplied by Dr. Ehrenstorfer (Ausburg, Germany) and Sigma-Aldrich (Barcelona, Spain). Standards of perfluoroalkyl substances (PFASs), Perfluorooctanoic acid (PFOA) and Perfluorooctane sulfonate (PFOS), were obtained from Wellington Laboratories (Guelp, ON, Canada) as methanol solutions at a nominal concentration of 50 μgmL-1. Each commercial standard of PFRs was weighed and dissolved in acetonitrile to obtain stock solutions of concentrations ranging from 2.0 to 4.0 mg mL^{-1}. For PFASs, individual stock solutions were prepared by diluting the commercial standard solution with acetonitrile to obtain 5.0 μg mL^{-1}. One of the individual mix working solutions was diluted with acetonitrile to obtain a concentration of 100 ng mL^{-1}.

Glacial acetic acid, ethanol absolute grade and methanol HPLC-M grade and Acetonitrile HPLC-MS grade were supplied by Merck (Darmstadt, Germany).

2.2. Sampling collection

A total of 18 plastic empty containers were provided by two food industries of the Valencian Region (Spain) during 2016: a) six Tetrabrick were destined to contain juice (with pH < 4.5) and juice with milk (with pH > 4.5); b) six pouches were destined to contain milk derivates, manufactured as infant food; and c) six bags to contain musts with fruit pulp (with pH > 4.5),

2.3. Sample preparation

Extraction of substances for the three studied materials was carried out in two different ways, standardised migration test (a) and destructive test (b).

(a) Standardised migration test

The migration test for the studied materials (bags, tetrabricks and pouches) was carried out following the European Union Regulation 10/2011. It is mandatory that this regulation be applied in plastic materials like bags cointaining musts with fruit pulp. Regarding pouches and tetrabrick materials, which are mainly made by multilayers, the regulation was applied because the inner layer was made of plastic.

Migration on tetrabricks and pouches was performed by filling them with simulant, because the internal part of the sample is destined to contain the food. Table 1 summarises the information about the simulant, the time and temperature applied in sample preparation for each matrix according to the Regulation 10/2011. Bags cointaining musts with fruit pulp were performed in steel stainless cells (Merck) where the inner layer was in contact with the simulant. In this case the layer

Table 1
Experimental conditions in migration tests.

Sample	Simulant	Temperature (°C)	Time (days)
Tetrabrick	Acetic acid 3% (B)	40	10
Tetrabrick	Etanol 50% (D1)	20	10
Pouches	Etanol 50% (D1)	20	10
bags	Etanol 50% (D1)	40	10

destined to contact with the food was extended in a stainless steel cell and put in contact with the simulant (Table 1).

For juice tetrabricks, simulant B (3% acetic acid in water, w/v) was used to simulate migration into acidic foods (fruit juices). Simulant D1 (50% ethanol in water v/v) is applied to study the migration into milk products and fruits derivates products. This simulant was applied to pouches (milk), to milk tetrabricks and bags cointaining musts with fruit pulp.

After migration, the simulant was collected in a Erlenmeyer flask with a glass stopper and stored at 4 °C until analysis.

(a) Destructive test

A non-standardized method was applied to identify substances present in one of the layers of the pouch, bags cointaining musts with fruit pulp and tetrabrick multilayered materials one. Destructive test is a term used to describe the non-standardized test that consists of breaking or destroying the entire container and not only the part in contact with the food

Tetrabricks, bags cointaining musts with fruit pulp and pouches were cut into small pieces (1 cm^2) and then all pieces of were soaked in 100 mL of 3% acetic acid in water, w/v, during 2 h at 70 degrees.

Next, the variously coloured liquids obtained from ink dissolution of acetic acid extraction were filtered and separated from the material pieces, after which 2 mL of dichloromethane (DCM) were added to 6 mL of the acidic extract.

The mixture was shaken and the inferior solution was transferred to another tube. Liquid–liquid extraction was completed twice. The extract was blown to dryness using nitrogen gas, and reconstituted with 200 µl of methanol- water (50:50, v/v).

2.4. UHPLC-Orbitrap-MS analysis

Table 2 describes the elemental composition, theoretical ion mass and fragment ion of target compounds (photoinitiators and PAAs). Chromatographic separation was performed on an Accela UHPLC system equipped with a Hypersil Gold aQ column (100 mm × 2.1 mm, 1.9 µm) both from ThermoFisher Scientific (Bremen, Germany). The flow rate used was 300 µl/min and the injection volume was 10 µl. Separations were performed using a binary gradient. The mobile phase was a gradient of H_2O (A) and methanol with 0.1% formic acid (B) and the binary gradient conditions were as follows: 0–8 min, linear from 2% to 50% B; 8–10 min, linear from 50% to 80% B; 10–11 min, linear from 80% to 95% B; 11–13 min isocratic 95% B; 13–15 min, linear from 95% to 2%. The total run time was 17 min.

Mass spectrometric analysis was performed on a single-stage Orbitrap MS (Exactive™, Thermofisher Scientific, Bremen, Germany). The system was equipped with a heated electrospray ionisation interface (HESI-II). The detection was carried out in positive ionisation mode (ESI+) in target analysis, and combining positive (ESI+) and negative (ESI-) in post-target analysis, using the following optimised operational parameters: spray voltage, 4.4 kV; sheath gas (N_2, > 95%), 50 arbitrary units (a.u.); skimmer voltage, 50 V; capillary voltage, 50 V; heater temperature, 500 °C; and capillary temperature, 123 °C. The mass spectra in target analysis was acquired using two alternating acquisition functions (i) full scan MS without fragmentation, ESI+; mass

Table 2
Elemental composition, monitored ion mass and fragment ions of the target compounds.

Compound	CAS-number	Elemental composition	Diagnostic ion	Theoretical monitored Mass (m/z) ion Da	Fragment ion 1 (m/z) ion/elemental composition	Fragment ion 2(m/z) ion/ elemental composition	RT Retention time (min)
1,5-Diaminonaphthalene (1,5 DAN)	2243-62-1	C10H10N2	$[M+H]^+$	159.09167	143.07287 (C10H9N)		3.6
3,3′ dimethylbenzidine (3,3′DMB)	119-93-7	C14H16N2	$[M+H]^+$	213.1386	181.0802 (C12H9N2)	196.1102 (C13H12N2)	5
1-Hydroxycyclohexyl phenyl ketone (HCPK)	947-19-3	C13H16O2	$[M+H]^+$	205.1223	186.9958 (C12H11O2)		3.5
2,2-dimethoxy-2-phenylacetophenone (DMPA)	24650-42-8	C16H16O3	$[M+H]^+$	257.11722	225.0910 (C15H13O2)		4.6
2,4-Diethyl-9H-thioxanthen-9-one (DETX)	82799-44-8	C17H16OS	$[M+H]^+$	269.09946	253.1045 (C17H17S)		9
2,4-Toluenediamine (2,4 TDA)	95-80-7	C7H10N2	$[M+H]^+$	123.09167	108.0682 (C6H8N2)	105.0573 (C7H7N)	2.4
2,6-Toluenediamine (2,6 TDA)	823-40-5	C7H10N2	$[M+H]^+$	123.09167	108.0682 (C6H8N2)	105.0573 (C7H7N)	1.8
2-Ethylhexyl 4-(dimethylamino)benzoate (EHDAB)	21245-02-3	C17H27NO2	$[M+H]^+$	278.21145	194.1175 (C11H16NO2)	166.0863(C9H12NO2)	8.9
2-Hydroxy-2-methylpropiophenone (HMPP)	7473-98-5	C10H12O2	$[M+H]^+$	164.08373	109.0647(C7H9O)		1.9
Isopropyl Thioxanthone (ITX)	5495-84−1	C16H14OS	$[M+H]^+$	255.08381	199.0575 (C13H11S)	213.0368 (C13H9O5)	8.1
4,4′-Bis(dimethylamino)-benzophenone (DEAB)	90–93-7	C21H28N2O	$[M+H]^+$	325.22744	297.1961 (C19H25N2O)	309.1961 (C20H25N2O)	7.2
Ethyl 4- dimethylaminobenzoate (EDMAB)	10287-53-3	C11H15NO2	$[M+H]^+$	194.11755	120.0808 (C8H10N)		4.6
4,4-Diaminodiphenyl ether (4,4′ DPE)	101-80-4	C12H12N2O	$[M+H]^+$	201.10223	108.0447 (C6H6ON)	184.0752 (C12H10ON)	4.9
4,4-Methylenedianiline (4,4′ MDA)	101-77-9	C13H14N2	$[M+H]^+$	199.12297	106.0652 (C7H8N)	76.0308 (C6H4)	5.3
4-Benzoylbiphenyl (PBZ)	2128-93-0	C19H14O	$[M+H]^+$	259.11174	181.0647 (C13H9O)		7.3
m-Phenylenediamine (m-PDA)	108-45−2	C6H8N2	$[M+H]^+$	109.07602	92.0495 (C6H6N)		1.3
Aniline (ANL)	62-53-3	C6H7N	$[M+H]^+$	94.05125	76.0308 (C6H4)		3.8
Benzophenone (BP)	119-61-9	C13H10O	$[M+H]^+$	183.08044	98.9844 (C8H2)		4.5

resolving power = 50,000 FWHM; scan range = 80–800 Da; scan time = 0.5 s (2 Hz); (ii) the same parameters but with full scan MS all ions fragmentation (the higher collision cell (HCD) was switched on at a collision energy of 20 eV). The automatic gain control (AGC) was set to 1×10^6 ions. The external mass calibration of the spectrometer was performed using a ready-to-use calibration mixtures (Mas Cal5 (+)) from Supelco (USA). Data acquisition and processing was performed using Thermo Scientific TraceFinder™ software version 3.1. Extracted ion chromatograms (XIC) for individual compounds were reconstructed from the full-scan data with a mass tolerance of 5 ppm.

2.5. Analytical method validation and identification criteria

The specificity of the method was tested by analysis of blank samples. We use this term for a simulant stored at the same conditions of time and temperature, without contact with the plastic material, than the migration test. The absence of chromatographic peaks at exactly the same retention time of the target compound indicated that no matrix compound were present to give a false positive signal. Calibration plots should show good linearity with correlation coefficients (R2) > 0.99.

The limit of quantification (LOQ) was established as the lowest concentration validated with satisfactory recovery (60–120%) and precision (CV < 25%). Precision was assessed as coefficient of variation CV (%), over 5 days.

Samples were analysed under quality assurance protocols following the ISO 17025. Quality control procedures to check the method performance were implemented in each batch of samples, including reagent blanks, blanks samples and spiked blank samples. Samples were stored at −4 °C until analysis. There is no reference material available on primary amines and photoinitiators in contact-food materials, so the accuracy of the method was carried out using a spiked blank of migration solution in two simulants B and D1 at three concentration levels. PAAs were fortified at these levels: 2 μg/kg^{-1}, 12.5 μg/kg^{-1} and 25 μg/kg^{-1}, except for ANL and 3,3′ DMB. Photoinitiators were validated for ITX and EHDAB at 0.5 μg/kg^{-1}, 25 μg/kg^{-1} and 200 μg/kg^{-1}. For the rest of photoinitiators 5 μg/kg^{-1}, 25 μg/kg^{-1} and 200 μg/kg^{-1} concentrations were validated. Six replicates at each level were injected within the calibration range of each analyte.

For the identification of analytes in the target quantitative method the following criteria were established: (i) mass accuracy of the molecular ion < 5 ppm; (ii) mass accuracy of the fragment ion (HCD) < 5 ppm and/or isotope pattern similar to the theoretical one; (iv) ion ratio similar to the standards with a relative tolerance of ± 30%, and (v) retention time similar to that of the calibration standard ± 0.2 min.

2.6. Database for post-run target screening analysis

An update of a previously generated theoretical database [3] was used. The current database contain 87 specific food-contact migrants was built grounded on the published literature [3,16,24]. Among the contaminants included in the database were phthalates, bisphenols, perfluorinated, phosphorus flame retardants and brominated compounds (Supplementary material, Table SI-1). For each substance, the screening database included the elemental composition and the theoretical accurate mass of the monitored molecular (quasi) ion. In this theoretical database no standards were used to get characteristic fragments [3,24]. Information about fragments was included when available in the literature, mainly from HRMS and QqQ (nominal mass) studies. When no fragments were found in the literature, those predicted by the software MassFrontier (version 7.0 from ThermoFisher) were included in the database. Likewise, the exact mass for all molecular and fragment ions was established and/or checked using both the MassFrontier and Xcalibur software (ThermoFisher).

To perform the screening and quantitative data analysis, the TraceFinder™ software (version 3.1, from ThermoFisher) was used. The identification and confirmation settings included a threshold override of 10,000, with S/N of 5 and a mass tolerance of 5 ppm for the molecular ion; an intensity threshold of 5000 and a mass tolerance of 5 ppm for the fragments. For the isotopic pattern a fit threshold of 90%, an allowed intensity deviation of 30%, and a mass deviation of 5 ppm were used. For quality control of the automated compound screening process made by the software, a few target blank and fortified samples were processed before and after the real samples.

2.7. Matrix effect

An extensive evaluation of the matrix effect using two simulants B and D1 was conducted based on the strategy developed by Matuszewsky. [25]. For this purpose, two sets of different simulants were prepared:

Set A. Standard solutions in simulants B and D1 at a concentration of 15 ng mL^{-1} Set B. Spiked blanks of simulants B and D1 at a concentration of 15 ng mL^{-1}

The Absolute Matrix Effect (ME_{abs}%) expressing the signal suppression or signal enhancement was investigated and calculated as:

$$ME_{abs}\% = \frac{A_{setB}}{A_{setA}} \times 100$$

where $A_{set\ A}$ and $A_{set\ B}$ are the peak areas obtained from the respective sets A and B

3. Results and discussion

3.1. Study of HCD fragmentation

High-energy collisional dissociation (HCD) is an efficient all fragmentation method that is widely used in Orbitrap mass spectrometers. The collision energy was varied in the range of 10–30 eV in this study, to select the most suitable collision energy to obtain the best response of the compound´s fragments.

As an example, Fig. 1 shows the response obtained for some target amines (ANL) and photoinitiators (DEAB and DETX) applying different collision energies. The response for Aniline was better at 30 eV, however, for other compounds, the optimised collision energy was 20 eV. Finally, a commitment collision energy of 20 eV was selected (only one value for all compounds could be selected). This collision energy provided good sensitivity for the majority of fragments.

3.2. Matrix effect

Matrix effect (ME) must be studied during method development to acknowledge the effect of the matrix over the ion suppression or the signal enhancement, which can modify the quantitative analysis. When the matrix effect is relevant, an appropriate calibration technique to compensate for this effect is necessary.

According to the procedure described previously (Section 2.9), the Absolute Matrix Effect (MEabs%) was calculated (Fig. 2) and classified into three different categories. There was no matrix effect when the ME percentage fell between 80% and 120%, because the repeatability of the results would be close to this range. A medium matrix effect was considered when the values ranged between 40% and 80% or 120% and 150%. A percentage below 40% or above 150% was classified as a high matrix effect.

The majority of amines showed ion suppression (Fig. 2). In simulant B (acetic acid 3%), 4,4′-DPE, 2,4-TDA, 2,6-TDA and 1,5-DAN presented moderated matrix effect with values of ME ranging from 60% to 80%. Others, such as aniline and m-PDA had high ion suppression, ranging from 20% to 50%. No matrix effect was observed in 4,4′-MDA and 3,3′-DMB. Regarding photoinitiators, BP and ITX presented moderate matrix effect with ME values ranging from 70% to 90% and whereas no matrix effect was observed in HMPP, HCPK, EDMAB, DMPA,PBZ, DEAB, DETX,

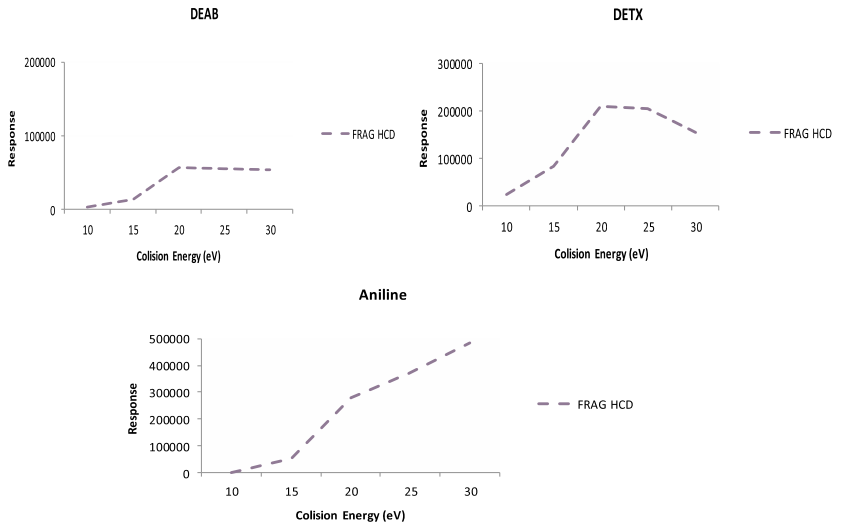


Fig. 1. Responses for molecular ion and fragments of DEAB, DETX and Aniline applying collision energies between 10 and 30 eV in HCD fragmentation.

and EHDAB. In simulant D1 (ethanol 50%),4,4′-DPE, 4,4′-MDA and 3,3′-DMB no matrix effect was observed. In the case of aniline, 2,4-TDA, 2,6-TDA and m-PDA presented moderate matrix effect with ME values ranging from 70% to 100%. Regarding all photoinitiators, no matrix effect was observed in simulant D1.

A compensation approach, such as the use of matrix-matched standards, is considered a useful method for minimising matrix effect consequences on data reliability (accuracy and precision). Consequently, a matrix-matched calibration curve was used for quantification. This was prepared spiking the blank sample with the standards

3.3. Analytical performance of the method for target analysis

A within-laboratory validation for the 18 total target analytes was performed to provide evidence of the method capacity for quantitative analysis of photoinitiators and amines in food simulants (see Table 3). Matrix-matched calibration plots showed good linearity with correlation coefficients (R2) > 0.99. The specificity of the method was tested by analysis of blank samples. The absence of extracted ion chromatographic peaks at exactly the same retention time of the target analytes indicated there were no matrix compounds giving a false positive signal in these samples.

Recoveries ranged between 72% and 120% for amines and photoinitiators in both simulants, with repeatability, in general, below 20%. The LOQ for photoinitiators was 0.5 μg/kg for ITX and EHDAB and 5 μg/kg, for the rest of photoinitiators. For PAAs, the LOQ was 2.5 μg/kg for ANL and 3.3′-DMB, and 2 μg/kg for the rest of the primary amines. The developed method meets the requirements of the EU regulation 10/2011 [7], and consequently could be used in the official

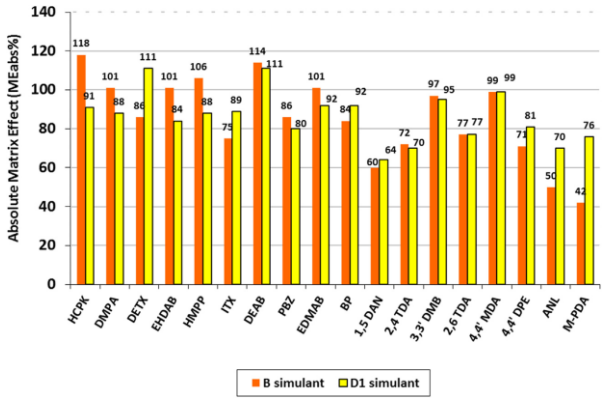


Fig. 2. Absolute matrix effect (ME_{abs} %) in different simulants.

Table 3
Quality parameters for the photoinitiators and primary aromatic amines in target analysis by migration test (n = 6).

		Levels in simulant B							
Photoinitiator	LOQ(μg/kg)	0.5 μg/kg simulant		5 μg/kg simulant		25 μg/kg simulant		200 μg/kg simulant	
		Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)
HMPP	5	–	–	78	2	80	8	88	12
HCPK	5	–	–	80	7	84	10	85	10
DMPA	5	–	–	82	4	85	13	90	9
DEAB	5	–	–	81	3	83	12	83	8
EHDAB	0.5	110	11	–	–	90	8	87	46
EDMAB	5	–	–	78	5	86	6	90	5
BP	5	–	–	80	8	89	8	91	8
PBZ	5	–	–	72	12	75	7	88	12
DETX	5	–	–	84	11	83	5	83	13
ITX	0.5	86	8	–	–	100	3	102	5
PAAs	LOQ(μg/kg)		2 μg/kg simulant		2.5 μg/kg simulant		12.5 μg/kg simulant		25 μg/kg simulant
		Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)
m PDA	2	110	13	–	–	90	4	95	7
2.6TDA	2	120	6	–	–	102	7	104	2
2,4TDA	2	118	11	–	–	90	10	103	7
1,5 DAN	2	102	13	–	–	85	4	92	4
4–4′ DPE	2	112	8	–	–	94	7	85	3
4,4′ MDA	2	85	7	–	–	99	3	102	2
ANL	2.5	–	–	89	15	89	15	94	15
3,3′ DMB	2.5	–	–	109	11	99	8	102	4
		Levels in simulant D1							
Photoinitiator	LOQ(μg/kg)	0.5 μg/kg simulant		5 μg/kg simulant		25 μg/kg simulant		200 μg/kg simulant	
		Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)
HMPP	5	–	–	80	8	85	9	86	2
HCPK	5	–	–	82	10	89	15	87	7
DMPA	5	–	–	78	13	78	12	85	4
DEAB	5	–	–	75	12	82	8	89	3
EHDAB	0.5	82	12	–	–	91	7	90	5
EDMAB	5	–	–	79	6	87	8	82	5
BP	5	–	–	80	8	84	9	84	8
PBZ	5	–	–	86	7	83	10	86	12
DETX	5	–	–	90	5	84	11	88	11
ITX	0.5	85	7	–	–	92	5	91	3
PAAs	LOQ(μg/kg)	2 μg/kg simulant		2.5 μg/kg simulant		12.5 μg/kg simulant		25 μg/kg simulant	
		Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)	Recovery(%)	CV(%)
m PDA	2	116	18	–	–	95	5	101	9
2.6TDA	2	119	8	–	–	100	5	103	7
2,4TDA	2	115	11	–	–	97	9	107	10
1,5 DAN	2	98	10	–	–	101	8	87	5
4–4′ DPE	2	116	15	–	–	95	7	95	4
4,4` MDA	2	101	12	–	–	99	3	102	2
ANL	2.5	–	–	87	15	78	15	92	15
3,3′ DMB	2.5	–	–	112	11	97	7	91	4

control.

In previous studies using LC-MS/MS, Sendom et al. [15] analysed PAAs with an LOQ 2.5 μg/kg. For ANL, 2,4 TDA, 4,4′ DPE and 4,4′ MDA. They only analysed amines in nylon kitchen utensils. Mattarozzi et al. [4] reported a lower LOQ than that in our study, but they only analysed amines in B simulant. Our method as a wider scope (Photoinitiators and amines) and was validated for two simulants. When a small number of substances are analysed, LC-MS/MS could give, in some applications, lower limits of detection than LC-HRMS, but the latter presents high selectivity (mass-resolving power, mass accuracy), and enough sensibility, and is a powerful analytical tool that can be used both, for target and non-target analysis ((retrospective screening analysis). There are no publications for amines in simulant D1.

There are no analytical methods published on photoinitiators in food simulants by LC –MS. ITX has a low LOQ for food samples [10] analysed with LC-MS/MS. For HCPK, DMPA, DETX, DEAB, PBZ and BP our LOQ is lower than those reported by Gallart-Ayala et al. [12] and Suciu et al. [13] which use GC-MS.

To our knowledge, there are no other works in the literature that combine in a single method the analysis of photoinitiators and amines with LC-HRMS in food contact materials

3.4. Target and post-target analysis of real samples

(a) Standardised migration test

Six bags cointaining musts with fruit pulp, six tetrabricks and six pouches were analysed using the standardised migration test following the Regulation 10/2011. No compound was found in migrated simulants. The absence of compounds like photoinitiators, perfluorinated compounds present in plastics or carton [8,11,19] could confirm that the application of an aluminium layer in tetrabricks and juice pouches can reduce or even eliminate the migration of these compounds into food or food simulants.

(a) Destructive test

Migration experiments with both simulants (B and D1) were performed for the multilayer materials in the three matrixes (bags cointaining musts with fruit pulp, pouches and tetrabricks). Multilayer includes the non-food contact side (outer side), the food contact side (inner side) and the barrier layers (aluminium). This was useful to study how the structure of the material and the application of layers can reduce, or even eliminate, migration of compounds into food or food simulants.

Table 4
Compounds detected in real samples by destructive test.

	Target			Post-target		
Compounds type	**Tetrabricks**	**Pouches**	**Bags**	**Tetrabricks**	**Pouches**	**Bags**
Photoinitiators	Benzophenone	EHDAB	–	–	–	–
PAAs	–	–	–	–	–	–
PFCs	–	–	–	PFOA/PFOS	PFOA/PFOS	PFOA/PFOS
PFRs	–	–	–	EHDPP/TCPP/TPhP	EHDPP/TCPP/TPhP	EHDPP

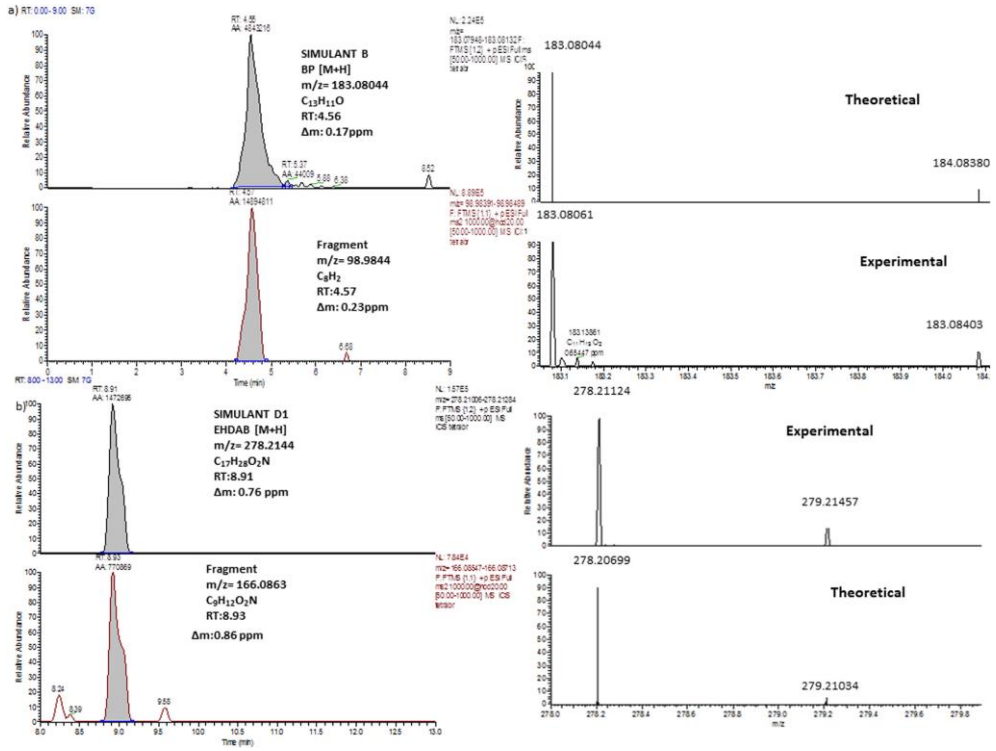


Fig. 3. Extracted ion chromatogram of the parent ions [M + H] and selected fragments along with experimental and theoretical isotopic pattern in target analysis for (a) BP, (b) EHDAB.

The multilayer migration only gave qualitative results because the extraction with DCM was not validated. Table 4 shows compounds detected in real samples by destructive test.

Referent to photoinitiators, Fig. 3 shows the extracted ion chromatograms of the two photoinitiators found in multilayer migration tests with simulant B and D1. Benzophenone and EHDAB compounds were found in migration solution from tetrabrick and pouch materials. However, they were not present in bags cointaining musts with fruit pulp. Gallart-Ayala et al. [24] also detected benzophenone in all carton materials at high concentrations (2 and 350 ng cm^{-2}) which contained various packaged foods such as fruit juices, baby food, water, wine and others. They also obtained EHDAB in many of the cartons analysed, similar to our study. In addition, they detected other photoinitiators such as PBZ, DEAB, 2-ITX, 4.ITX, DETX, DMPA and EDMPA that were not found in our study. Until now, a maximum permitted amount for migration from packaging materials to packaged food has only been established for benzophenone [24]. Benzophenone was also detected by Vavrous et al. (2016) in coated bakery release papers for oven baking at temperatues up to 220 °C with maximum concentrations of 18 mg/kg [8].

In post-target analysis two polyfluorinated compounds (PFCs), PFOA and PFOS were identified in tetrabricks, pouches and bags cointaining musts with fruit pulp in our study. Fig. 4 shows the extracted ion chromatogram of PFOA in juice pouches.

Shoeib et al. [26] also detected PFOA in 79% of food packaging material, processed and unprocessed cardboard pasta containers and unprocessed baking cups with median concentration of 2.40 ng g^{-1}. High PFOA values of 65 and 94 ng g^{-1} were exhibited in two food-

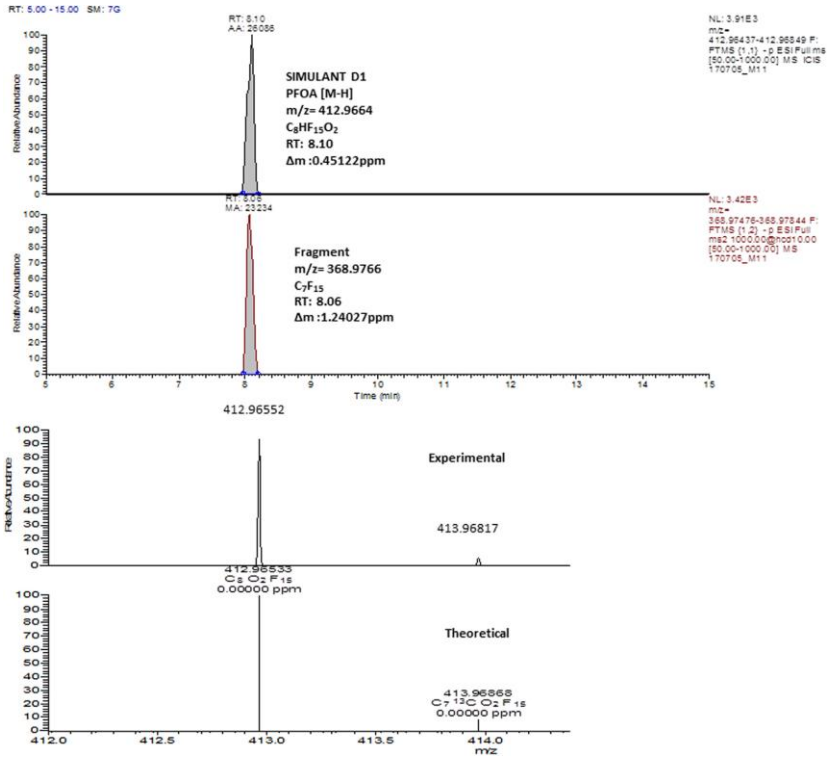


Fig. 4. Extracted ion chromatogram of the parent ions [M-H] and selected fragments along with experimental and theoretical isotopic pattern in post-run target analysis for PFOA.

paper wrapping samples [26]. Likewise, Kotthoff et al. reported PFOA in paper-based food contact from Germany, with a high concentration, of 658 ng g^{-1}[27]. Microwaveable popcorn bags has been the subject of many investigations; Begley et al. reported PFOA concentrations between 6 and 290 ng g^{-1} [28] Another study found 9.2 ng g^{-1} in microwaveable popcorn bags, while no PFOA was detected in the popped popcorn itself after microwaving [29].

Phosphorus flame retardants (PFRs) were also identified in the post-target analysis. Fig. 5 presents the extracted ion chromatogram for EHDPP in juice tetrabrick. Three PFRs (EHDPP, TCPP and TPhP) were found in tetrabricks and juice pouches, and one PFRs (EHDPP) was present in bags containing musts with fruit pulp migrated solution. To our knowledge, there are no results of PFRs in food contact materials in the literature. Some studies say that PFRs concentrations in foods were not significantly affected by the packaging materials [30]. Zhang et al. investigated the concentrations of 6 typical PFRs in 50 rice samples. The concentrations of ΣPFRs in foods ranged from 0.004 ng/g to 287 ng/g. The highest PFRs concentrations were found in rice and vegetables. TCPP predominated in most food samples [30]. EHDPP was described in most food categories in a Swedish food market basket study, with the highest median concentrations in pastries (9 ng/g). EHDPP was followed by TPhP (2.6 ng/g in fat/oils) and TCPP (1.0 ng/g in fat/oils) [31].

In our study, inks, perfluorinated and organophosphate flame-retardant compounds were detected in multilayer migration tests and were not observed in the inner layer and filling conditions tests described in the Regulation. Therefore, the absence of the aforementioned compounds in food simulants could confirm that the application of an aluminium layer can reduce or even eliminate the migration of these components to food.

In addition, the use of acetic acid 3% as an extraction solvent, before the addition of dichloromethane, has provided better results rather than the use of only DCM solvent. When the extraction was carried out only with DCM more compounds were extracted producing analytical interferences in the destructive sample preparation.

Aznar et al. [32] studied migration of inks components from 3 different sets of materials in order to study how the structure of the multilayer materials and the materials used could affect final migration of ink components to food. When there were no layers covering inks, even if inks were applied in the non-food side of the packaging, intense migration was found. The effect of having two layers separating the ink was tested, in order to avoid or delay the migration process. In both cases the migration decreased when they were used, especially with aluminium. Despite the use of these layers, some ink components were present in the migration. When aluminium was used, the concentration of most ink migrants decreased, and 5 out of the 9 studied compounds even disappeared [32].

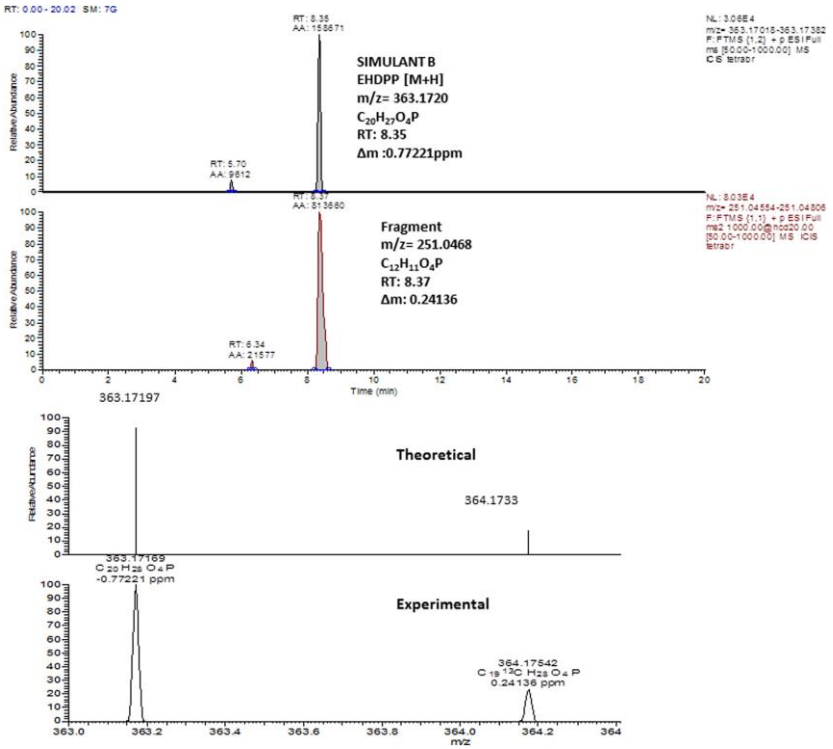


Fig. 5. Extracted ion chromatogram of the parent ions [M + H] and selected fragments along with experimental and theoretical isotopic pattern in post-run target analysis for EHDPP.

4. Conclusions

An analytical strategy based on liquid chromatography high resolution mass spectrometry for the quantitative target analysis of ten UV-ink photoinitiators and eight PAAs in bags cointaining musts with fruit pulp, juice tetrabricks and juice milk pouches have been developed and applied to 18 packaging samples.

To our knowledge, this methodology is the only one published than combines photoinitiators with amines in LC-HRMS in food simulants.

The analytical potential of the high-resolving power, the accurate mass and the acquisition in full scan (with and without fragmentation), enables the use of a retrospective analysis using an extensive database of 87 analytes.

In our opinion, this analytical approach, which could be improved with more fragment data on the screening database, is nevertheless a promising tool for a more extensive control of packaging and food in contact with materials.

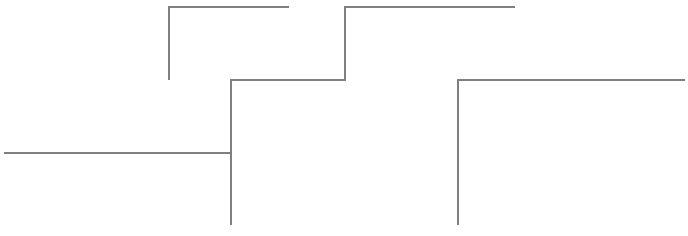
Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.08.047.

References

[1] European Printing Ink Association, EUPIA, Inventory list comprising packaging ink raw materials applied to the non-food contact surface of food packaging, 2013.
[2] M. Aznar, C. Domeño, C. Nerín, O. Bosetti, Set-off of non volatile compounds from printing inks in food packaging materials and the role of lacquers to avoid migration, Dyes Pigments 114 (2015) 85–92.
[3] Y. Sanchis, C. Coscollà, M. Roca, V. Yusà, Target analysis of primary aromatic amines combined with a comprehensive screening of migrating substances in kitchen utensils by liquid chromatography-high resolution mass spectrometry, Talanta 138 (2015) 290–297.
[4] M. Mattarozzi, F. Lambertini, M. Suman, M. Careri, Liquid chromatography–full scan-high resolution massspectrometry-based method towards the comprehensive analysis of migration of primary aromatic amines from food packaging, J. Chromatogr. A 1320 (2013) 96–102.
[5] D. Pezo, M. Fedeli, O. Bossetti, C. Nerón, Aromatic amines from polyurethane adhesives in food packaging: the challenge of identification and pattern recognition using Quadrupole-Time of Flight- Mass Spectrometry, Anal. Chim. Acta 756 (2012) 49–59.
[6] C. Brede, I. Skjervak, H. Herikstad, Determination of primary aromatic amines in water food simulant using solid-phase analytical derivatization followed by gas chromatography coupled with mass spectrometry, J. Chromatogr. A 983 (2003) 35–42.
[7] European commission, CommissionRegulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Off. J.Eur. UnionL12, 2011, p. 1.
[8] L.A. Vavrous, J. Vapenka, K. Sosnovcov, K. Kejlov, D. Vrbík Jírov, Method for analysis of 68 organic contaminants in food contact paper using gas and liquid chromatography coupled with tandem mass spectrometry, Food Control. 60 (2016) 221–229.
[9] Y. Sanchis, V. Yusà, C. Coscollà, Analytical strategies for organic food packaging contaminants, J. Chro-matogr. A 1490 (2017) 22–46.

[10] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Analysis of UV ink photoinitiators in packaged food by fast liquid chromatography at sub-ambient temperature coupled to tandem mass spectrometry, J. Chro-matogr. A 1218 (2011) 459–466.
[11] D. Shen, H. Lian, T. Ding, J. Xu, C. Shen, Solid-phase microextraction followed by gas chromatography–mass spectrometry for the determination of ink photo-initiators in packed milk, Anal. Bioanal. Chem. 395 (2009) 2359–2367.
[12] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Solid-phase microextraction followed by gas chromatography–mass spectrometry for the determination of ink photo-initiators in packed milk, J. Chro-matogr. A 1208 (2008) 182–200.
[13] N.A. Suciu, F. Tiberto, S. Vasileiadis, L. Lamastra, M. Trevisan, Recycled paper-paperboard for food contact materials: contaminants suspected and migration into foods and food stimulant, Food Chem. 141 (2013) 4146–4415.
[14 M. Aznar, A. Rodriguez-Lafuente, P. Alfaro, C. Nerin, UPLC-Q-TOF-MS analysis of non-volatile migrants from new active packaging materials, Anal. Bioanal. Chem. 404 (2012) 1945–1957.
[15] R. Sendom, J. Bustos, J. Sanchez, P. Paseiro, Me Cirugeda, Validation of a liquid chromatography–mass spectrometry method for determining the migration of primary aromatic amines from cooking utensils and its application to actual samples, Food Addit. Contam. Part A 27 (2010) 107–117.
[16] H. Gallart-Ayala, O. Nunez, P. Lucci, Recent advances in LC-MS análisis of food-packaging contaminants, Trends Anal. Chem. 42 (2013) 99–124.
[17] M.M. Gómez-Ramos, C. Ferrer, O. Malato, A. Agüera, A.R. Fernández-Alba, Liquid chromatography-high-resolution mass spectrometry for pesticide residue analysis in fruit and vegetables: screening and quantitative studies, J. Chro-matogr. A 1287 (2013) 24–37.
[18] L. Polgár, J.F. García-Reyes, P. Fodor, A. Gyepes, M. Dernovics, L. Abrankó, B. Gilbert-López, A. Molina-Díaz, Retrospective screening of relevant pesti-cide metabolites in food using liquid chromatography high resolution massspectrometry and accurate-mass databases of parent molecule and diagnosticfragment ions, J. Chromatogr. A 1249 (2012) 83–91.
[19] C. Moreta, M.T. Tena, Determination of perfluorinated alkyl acids in corn, popcorn and popcorn bags before and after cooking by focused ultrasound solid–liquid extraction, liquid chromatography and quadrupole-time of flight mass spectrometry, J. Chromatogr. A 1355 (2014) 211–218.
[20] C. Bignardi, A. Cavazza, C. Corradini, P. Salvadeo, Targeted and untargeted data-dependent experiments for characterization of polycarbonate food-contact plastics by ultra high performance chromatography coupled to quadrupole orbitrap tandem mass spectrometry, J. Chromatogr. A 1372 (2014) 133–144.
[21] W. Jia, X. Chu, Y. Ling, J. Huang, J. Chang, Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry, J. Chromatogr. A 1362 (2014) 110–118.
[22] N. León, A. Pastor, V. Yusà, Target analysis and retrospective screening of veterinary drugs, ergot alkaloids, plant toxins and other undesirable substances in feed using liquid chromatography–high resolution mass spectrometry, Talanta 149 (2016) 43–52.
[23] C. Coscollà, N. León, A. Pastor, V. Yusà, Combined target and post-run target strategy for a comprehensive analysis of pesticides in ambient air using liquid cromatography- Orbitrap high resolution mass spectrometry, J. Chromatogr. A 1368 (2014) 132–142.
[24] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Fast liquid chromatography- tandem mass spectrometry for the analysis of bisphenol A-diglycil ether, bisphenol F-diglycidyl ether and their derivates in canned food and bevereges, J. Chromatogr. A 1218 (2011) 1603–1610.
[25] B.K. Matuszewsky, M.L. Constanzer, C.M. Chavez-Eng, Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS, Anal. Chem. 75 (2003) 3019–3030.
[26] T. Shoeib, Y. Hassan, C. Rauert, T. Harner, Poly-and perfluoroalkyl substances (PFASs) in indoor dust and food packaging materials in Egypt: trends in developed and developing countries, Chemosphere 144 (2016) 1573–1581.
[27] M. Kootthoff, J. Muller, H. Jürling, M. Sclummer, D. Fiedler, Per-and poly-fluoroallkyl substances in consumer products, Environ. Sci. Pollut. Res (2017), https://doi.org/10.1007/s11356-015-4202-7 (online 20).
[28] T.H. Begley, W. Hsu, G. Noonan, G. Diachenco, Migration of fluorochemical paper additives from food-contact paper into foods and food simulants, Food Addit. Contam. 25 (2008) 384–390.
[29] S. Dolman, M. Pelzing, An optimized method for the determination of per-fluorooctanoic acid, perfluorooctane sulfonate and other perfluorochemicals in different matrices using liquid chromatography/ ion-trap mass spectrometry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (2011) 2043–2050.
[30] X. Zhang, W. Zou, L. Mu, Y. Chen, Ch Ren, X. Hu, Q. Zhou, Rice ingestion is a major pathway for human exposure to organophosphate flame retardants (OPFRs) in China, J. Hazard. Mater. 318 (2016) 686–693.
[31] P. Giulia, A. Glynn, G. Malarvannan, A. Covaci, Dietary intake of phosphorus flame retardants (PFRs) using Swedish food market basket estimations, Food Chem. Toxicol. 100 (2017) 1–7.
[32] M. Aznar, P. Alfaro, C. Nerín, E. Jones, E. Riches, Progress in mass spectrometry for the analysis of set-off phenomena in plastic food packaging materials, J. Chromatogr. A 1453 (2016) 124–133.



Capítulo 3

4.3. Capítulo 3. Análisis de 4 Parabenos (MP, EP, PP, BP) y Bisfenol A, F y S en orinas, utilizando dilución y análisis directo mediante LC-MS/MS.

4.3.1. Resumen

Los bisfenoles (BPs) y parabenos son sustancias químicas muy utilizadas en la fabricación de diversos productos de consumo en todo el mundo (Geens et al. 2011, Geens et al. 2012). El plastificante BPA, o 2, 2 (4, 4-dihidroxidifenilo) propano, es utilizado por los fabricantes industria para la producción de policarbonato, resinas epoxi, retardantes de llama, y otros productos (selladores dentales y productos de cuidado personal). También productos que incluyen adhesivos, recubrimientos, protectores y en envases de alimentos (Mallozzi et al. 2016, Vandenberg, Hauser et al. 2007, Staples et al. 1998). La principal fuente de exposición de la población a BPA es la ingestión de alimentos y productos alimentarios envasados.

Aunque el BPA puede utilizarse en materiales en contacto con alimentos en la Unión Europea (UE), según el Reglamento 10/2011, con un límite de migración específico máximo (LME) de 0,6 $mg \cdot kg^{-1}$ (EU 10/2011), el uso de BPA en la fabricación de botellas de policarbonato para lactantes ha sido prohibida por la Comisión Europea (CD 2011/8/EU) y esta sustancia está incluida en el REACH por sus propiedades como disruptor endocrino (Mallozzi et al. 2016, Vandenberg et al. 2007) y por su toxicidad en la reproducción (ECA 2017). Las recientes limitaciones en el uso de BPA han favorecido el uso de compuestos análogos como bisfenol F (BPF) y S (BPS) (Rochester 2015). Sin embargo, algunos estudios sugieren que el BPF y el BPS muestran efectos endocrinos similares a los del BPA (Rochester 2015, Wu et al. 2018) y se han incluido en la lista de sustancias prioritarias en el proyecto Human Biomonitoring for Europe (HBM4EU 2017).

Después de la ingestión, el BPA se metaboliza y se excreta, principalmente en la orina, con una vida media de <6 h. El metabolismo incluye la glucuronización o la sulfatación, para aumentar su solubilidad en agua (Mattison et al. 2014). El metabolismo y la eliminación de BPF y BPS es menos conocido, aunque la literatura sugiere que es similar al BPA (Rochester 2015). Los niveles en orina de bisfenoles libres y totales (conjugados + no conjugados), se usan comúnmente como biomarcadores de exposición reciente (Moos et al. 2014).

Los ésteres alquilo del ácido p-hidroxibenzoico (parabenos) son un grupo de productos químicos utilizados como conservantes en productos cosméticos, farmacéuticos y en alimentos (Darbre 2008, Beldzka et al. 2014). La ingesta de alimentos ha sido considerada como un aporte diario de parabenos, por ello la Autoridad Europea de Seguridad Alimentaria realizó una evaluación de riesgo en 2004 (EFSA 2004a) y estableció unos niveles de ingesta diaria admisible (IDA) de 10 mg / kg de peso corporal / día para el MP y EP, pero no se establecieron niveles para otros parabenos.

El Reglamento (CE) no 1223/2009 de la Unión Europea sobre los productos cosméticos (EU 2009) y sus modificaciones, permiten el uso de cuatro parabenos (y sus sales) en productos de cuidado personal. La principal vía de exposición a parabenos es la absorción dérmica. Después de ser absorbidos por la piel, los parabenos son metabolizados por las esterasas y / o conjugados y excretados en la orina y la bilis en forma de glucurónicos (Boberg et al. 2010). Algunos estudios han encontrado una posible relación entre la exposición a parabenos y las actividades estrogénicas y anti androgénicas (Boberg et al. 2010, Darbre 2008). Por ello, es importante la realización de estudios de biomonitorización en humanos, como herramienta eficaz y necesaria para evaluar la exposición interna a sustancias químicas. Estos programas de biomonitorización requieren métodos analíticos apropiados (Vandenberg et al. 2007, Beldzka et al. 2014, Yusa et al. 2012).

Los metabolitos de parabenos y bisfenoles (libres, conjugados) en orina son los biomarcadores que proporcionan información sobre la exposición interna reciente para este tipo de sustancias. La tabla 18, recoge los métodos analíticos recientes descritos en la literatura para la determinación de estas sustancias en orina.

Estudios anteriores a nivel nacional e internacional, reflejan unos niveles de BPA entre 0.2 - 30ng·mL^{-1}, con una frecuencia de detección superior al 40% (Geens et al. 2014, Yang et al. 2014, Moos et al. 2014, Heffernan et al. 2016, Jiménez-Díaz et al. 2016, ZHANG et al. 2016). Los niveles de parabenos están en el rango de 0.1 a 1000 ng mL^{-1}, siendo el MP el que presenta mayor frecuencia de detección ($\geq$ 75%) en muestras de orina (Moos et al. 2014, Jardim et al. 2015, Moos et al. 2015, Jiménez-Díaz et al. 2016).

La presencia en la orina a niveles traza y la complejidad de la matriz requieren el uso de metodologías analíticas sensibles y selectivas. Además, la gran cantidad de muestras que generalmente se requieren en los programas de biomonitorización hacen necesario el

desarrollo de técnicas multi residuos para la determinación de varios contaminantes en el mismo análisis.

En este trabajo se ha desarrollado una nueva estrategia analítica basada en "*dilute and shoot*" (diluir e inyectar) y LC-APCI-MS/MS para la determinación simultánea de Bisfenoles (A, F, S) y Parabenos (metil, etil, propil, butil parabeno) en muestras de orina humana.

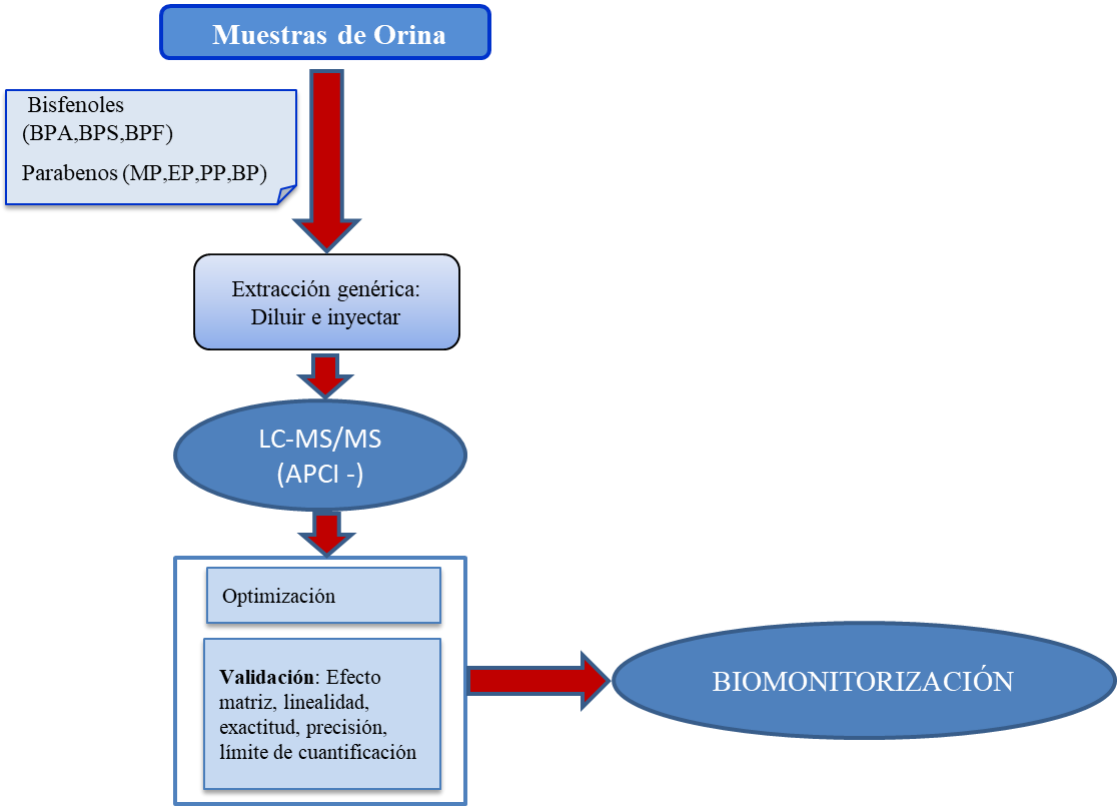


Figura 19. Estrategia de análisis para la determinación de bisfenoles y parabenos en orina humana.

Tabla 18. Revisión de las metodologías analíticas para la determinación de bisfenoles y parabenos en orina

Referencia	Analitos	Volumen muestra (µL)	Preparación de muestra	Equipo	Fuente de ionización	Calibración	LD (ng/mL)	LC (ng/mL)
(Heffernan et al. 2016)	BPA, BPB, BPS, BPAF, BPF, MMP, MEP, MiBP, MCHP, MBP, MBzP, MEHP, MEOHP, MEHHP, MECPP, MOP, MCPP, MNP, MDP	50	Hidrólisis enzimática + 400 µL de ácido fórmico 0.5 % SPE-LC-MS/MS (Strata X 20x2.0 mm, 25 µm phenomenex)	LC-QTRAP-MS/MS con Synergi MAX-RP 150×3.00 mm, 4 µm	ESI (-)	Calibración sobre matriz (orina sintética)	BPA 0.10 BPS 0.067 BPB 0.26 BPF 0.39	-
(Venisse et al. 2014)	BPA y derivados	300	Extracción líquido-liquido (con formiato amónico 10M)	UHPLC - Acquity H Class, acoplado a Xevo TQ-SQ con columna ACQUITY CSH C18 (1.7 µm tamaño de particula, 2.1×100 mm)	ESI (-)	Calibración sobre matriz	-	0.5
(Jardim et al. 2015)	MP,EP,PP,BP,ByP	200	Microextracción con sorbentes	UHPLC-MS/MS con columna Kinetex C18 (100 mm × 2.1 mm × 1.7 µm)	ESI (-)	Calibración externa	-	0.5
(Yang et al. 2014)	BPA, BPS, BPF, BPAF	2000	Hidrólisis enzimatica + Extracción liuqido-liquido con atilacetato	UHPLC-MS/MS Acquity BEH columna C18 (2.1 mm × 100 mm; 1.7 µm;	ESI (-)	Calibración externa	BPS 0.010 BPF 0.10 BPA 0.09 BPAF 0.008	BPS 0.032 BPF 0.31 BPA 0.27 BPAF 0.024

(Rocha et al. 2018)	BPA, BPS, BAP, BPP, , BPAF, BPZ), 7 parabenos (methyl-, ethyl-, propyl-, butyl-, benzyl-paraben, methyl-protocatechuic acid, y ethyl-protocatechuic acid), 5 benzophenones (benzophenone-1, benzophenone-2, benzophenone-3, benzophenone-8, and 4-hydroxybenzophenone), triclosan y triclocarban	5000	Hidrólisis enzimatica + Micro extracción liquido-liquido (con corriente de aire)	LC-MS/MS con Atlantis T3 columna C18 (75 mm × 2.1 mm i.d. y 3.0 µm)	ESI (-)	Calibración sobre matriz	BPF 0.25	BPS 0.07 BPF 0.25 BPA 0.1 MP 0.05 EP 0.05 PP 0.1 BP 0.1
(Moos et al. 2014)	MP, EP, PP, BP, BPA, triclosan y benzofeona	300	Hidrólisis enzimatica + congelación	LC-MS/MS con 2 columnas : RAM fase (LiChrospher® RP-8 ADS (25 µm) 25 mm × 4 mm RAM from Merck, Darmstadt, Germany), la separación cromatografica hecha con C18 (Atlantis dC18 30 mm × 150 mm; 3 µm, Waters)	ESI (-)	Calibración con patrones preparados sobre agua	-	-
(Zhao et al. 2018)	Bisfenoles (BPA, BPS, BPF, BPP, BPZ, BPAP), benzofenonas y parabenos (MP,EP,PP,BP,BzP)	1000	Enzymatic hydrolysis + extracción liuqido-liquido	UHPLC-TQMS con Scientific Betasil C18 columna (2.1 mm 100 mm,3 µm)	ESI (-)	Calibración externa	BPS 0.2 BPA 0.2 MP 0.05 EP 0.05 PP 0.1 BP 0.1	-
(Jiménez-Díaz et al. 2016)	MP, EP, PP, BP y BPA	-	Enzymatic hydrolysis + Microextracción liquido-liquido en fase dispersa (DLLME)	UHPLC-MS/MS con C18 (50 mm × 2.1 mm I.D., 1.7 µm) de Waters	ESI (-)	Calibración sobre matriz	-	0.5

4.3.2. Discusión de resultados

a. Optimización de la fuente de ionización

La ionización por electroespray (ESI) es la técnica más utilizada en el análisis de bisfenoles y parabenos en orina mediante cromatografía líquida (tabla 18). Sin embargo, otros autores señalan el uso de APCI para la determinación de estos compuestos (Sanchis et al. 2017, Gallart-Ayala et al. 2013). En el presente trabajo, con el objetivo de establecer la fuente de ionización óptima en base a la respuesta de los compuestos, se inyectó una solución patrón que contenía todos los compuestos por sextuplicado en ambas fuentes ESI (-) y APCI (-), obteniendo una señal diez veces mayor para BPA y BPS en la fuente APCI (-). El resto de compuestos (MP, EP, PP, BP y BPF) presentaron una respuesta de señal similar en ambas fuentes. En consecuencia, se seleccionó APCI (-) como la fuente de ionización.

Para optimizar la eficiencia de la fuente de ionización, se llevó a cabo un diseño estadístico de experimentos (DoE), utilizando el software MINITAB. De acuerdo con la literatura (Mei et al. 2003), los principales factores que afectan a la fuente de iones APCI y por tanto los parámetros estudiados fueron la presión del gas envolvente (SGP), la temperatura capilar (CT), el flujo de gas auxiliar (AG), la corriente de descarga (DC) y la temperatura de vaporización (VT). Seguidamente mediante un diseño central compuesto (CCD), se optimizaron los valores de los parámetros significativos, obteniendo los valores reflejados en la tabla 19.

Tabla 19. Valores optimizados de los parámetros significativos de la APCI

	VT (ºC)	CT (ºC)	DC (µA)	SGP (psi)	AG (a.u.)
Rango estudiado	100-500	70-350	1-10	5-60	0-20
Valor óptimo	400	250	4	45	4

Tube lens offset 163V, Colission energy -10 eV

b. Separación Cromatográfica

Para obtener una buena separación cromatográfica se probaron diferentes columnas y fases móviles. En la literatura, se utilizan varios modificadores para la determinación de parabenos y fenoles en la orina con fuente ESI (Moos et al. 2014, Heffernan et al. 2016),

en cambio hay poca información respecto a la cromatografía con fuente APCI para estas sustancias; después de múltiples experimentos, se seleccionó una fase móvil compuesta de agua (a) y metanol (B) como óptima. El uso de esta fase móvil produjo la mejor respuesta para los iones padre y producto seleccionados en APCI (-). Diferentes ensayos realizados en el presente trabajo utilizando fases móviles con ácido acético producían una señal baja en los bisfenoles (A, F, S), probablemente este hecho se deba a la inhibición de la ionización.

Después de probar distintas columnas, la mejor separación cromatográfica se obtuvo con la columna Luna C18 (2) (150 mm x 2.00 mm de diámetro interno, tamaño de partícula 5 µm).

c. Optimización de la extracción de muestra

De los métodos de extracción probados para la determinación de los bisfenoles y parabenos, tanto el método basado en la extracción QuEChERS cómo el de diluir e inyectar directamente dieron buenos resultados. De las modificaciones del método QuECHERS ensayadas, la que resultó más apropiada fue la que comparte el uso de las sales de extracción (4g $MgSO_4$ +1g NaCl +1g Na citrato +0.5g SCDS), seguida de la concentración en corriente de nitrógeno del extracto orgánico. Con este método se pudieron detectar la totalidad de los analitos, con una sensibilidad apropiada, siendo innecesaria una etapa posterior de *clean-up* con las habituales fases dispersas de extracción en fase sólida. Sin embargo, comparando con la dilución e inyección directa no mejoró la sensibilidad, obteniendo una sensibilidad adecuada para llegar al límite de cuantificación con ambos métodos de extracción, por lo que se optó por esta segunda forma de preparación de muestra a fin de reducir los tiempos y los costes de la preparación.

d. Estudio del efecto matriz y validación del método analítico

El estudio del efecto matriz se hizo según el método descrito por Delma et al. (2018). La evaluación del efecto matriz en orina humana se realizó en todo el rango de calibración comparando las pendientes de la curva con adición de patrones en agua y/o orina sintética y la curva en matriz de orina humana. Para este propósito, previamente, se cuantificó la concentración existente en la orina humana utilizando una curva de calibrado preparada con orina sintética en todo el rango de concentración (de 0,2 a 300 ng mL^{-1}). Cada área

del pico también se normalizó al área del pico de cada IS para cada muestra. Se aplicó la ecuación para la pendiente:

% Efecto matriz = ((pendiente MM − pendiente MS) / pendiente MS)) x 100

donde MM es matriz en muestra y MS es la matriz en estándares (en agua u orina sintética).

El efecto matriz (ME) se calculó para todos los compuestos y los resultados mostraron la ausencia del efecto de la matriz (<20%). Para los parabenos (MP, EP, PP y BP), el ME calculado fue -7, -18, -6 y -15% y para bisfenoles (BPA, BPF y BPS), -7, -18 y -19% respectivamente. Esta ausencia de efecto matriz < 20% puede explicar por qué se empleó la dilución de la muestra y es una buena opción para evitar la supresión. Además, la fuente de APCI generalmente se considera menos afectada por los efectos de la matriz debido a su diferente modo de ionización; en este caso la ionización tiene lugar en la fase gaseosa (Mei et al. 2003). En las figuras 20 y 21 se puede observar el estudio del efecto matriz para el BPA y MP.

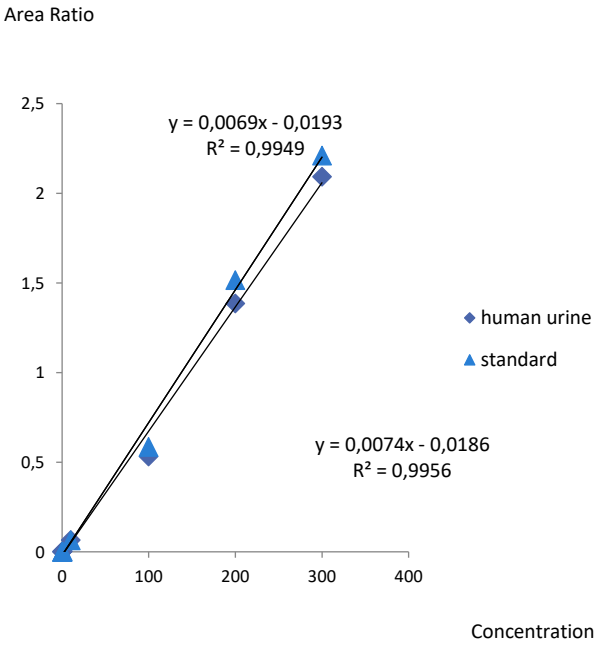


Figura 20. Estudio del efecto matriz para el BPA.

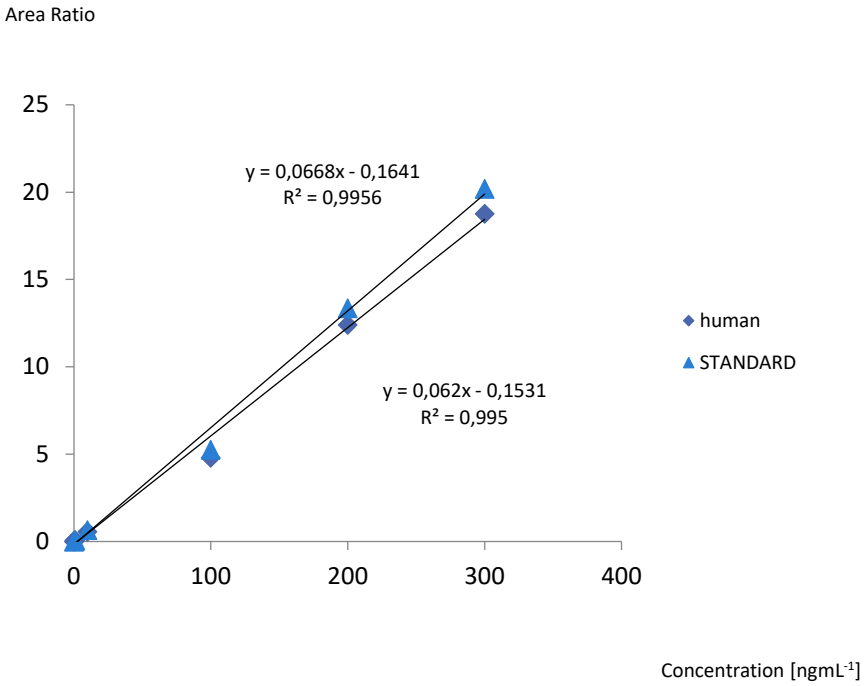


Figura 21. Estudio del efecto matriz para el MP

Por tanto, en este trabajo dada la ausencia del efecto matriz, se usó tanto agua como orina sintética, con adición de estándares en la calibración y se emplearon muestras de orina humana enriquecidas para los controles de calidad.

La linealidad del método se evaluó en dos rangos de concentración (de 0,2 a 10 ng mL^{-1} y de 10 a 300 ng mL^{-1}). La precisión del método se evaluó utilizando una muestra de orina humana enriquecida (mezcla de distintas orinas blancas) a cuatro niveles de concentración (0,2, 10, 100 y 300 ng mL^{-1}). En la figura 22 se puede observar una muestra de orina humana adicionada al límite de cuantificación. La precisión se evaluó como coeficiente de variación (%), en 5 días.

El límite de cuantificación (LC) se estableció como la concentración más baja validada con una recuperación y precisión satisfactorias, siendo 0.2 ng mL^{-1} el LC para todas las sustancias (MP, EP, PP, BP, BPA, BPF y BPS) con recuperaciones desde el 85% del BP al 107 % para el PP y precisión desde el 2% del EP al 15 % para el BP o el BPS. Los criterios de recuperación aceptables fueron de 70% a 120% y precisión CV <20% (SANTE/11813/2017). Se inyectó un blanco de reactivo en cada serie de trabajo para controlar la posible contaminación.

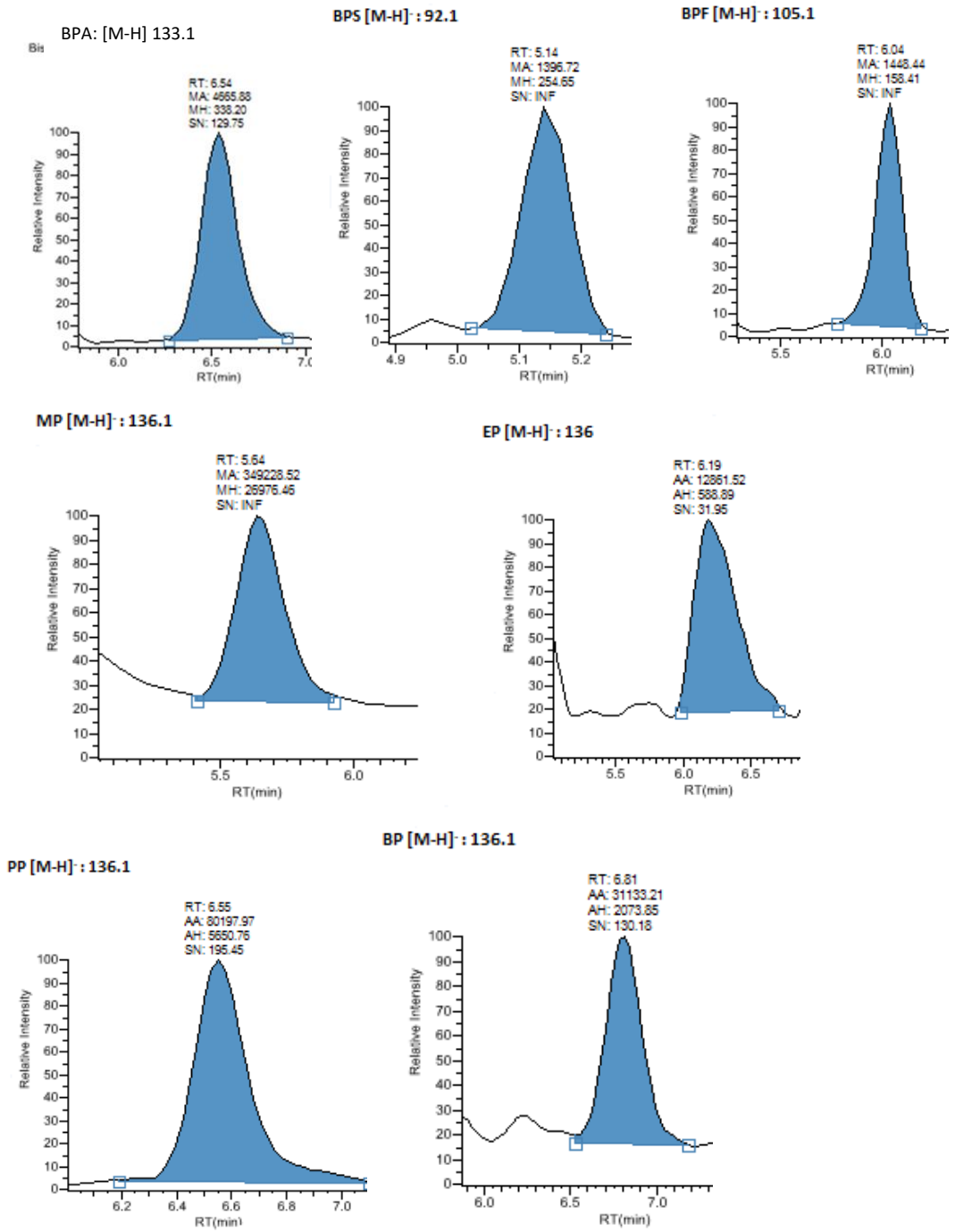


Figura 22. Cromatograma del ion diagnóstico del MP, EP, PP, BP, BPA, BPF y BPS en una muestra de orina humana adicionada al LC (0.2 ng mL^{-1}).

e. Aplicación a muestras reales

El análisis fue aplicado satisfactoriamente a 15 muestras de orina. El MP y el BPA fueron las sustancias detectadas con mayor frecuencia, el 93% de las muestras presentaba niveles de MP y el 80% en el caso del BPA. El MP cuantificado con valores desde 0.2 ng mL^{-1} a 2952 ng mL^{-1}, y el BPA desde 0.2 ng mL^{-1} a 12 ng mL^{-1}.

EP se encontró con una frecuencia de detección del 60% y concentraciones desde el LC hasta 9 ng mL^{-1}. PP, BP, BPS y BPF se detectaron con menor frecuencia FD < 40% y con concentraciones hasta 4.7 ng mL^{-1} para PP, 0.5 ng mL^{-1} para BP, 39 ng mL^{-1} BPS y 8.5 ng mL^{-1} para BPF. La figura 23 corresponde a una muestra de orina en la que se han cuantificado BPA, BPF y MP.

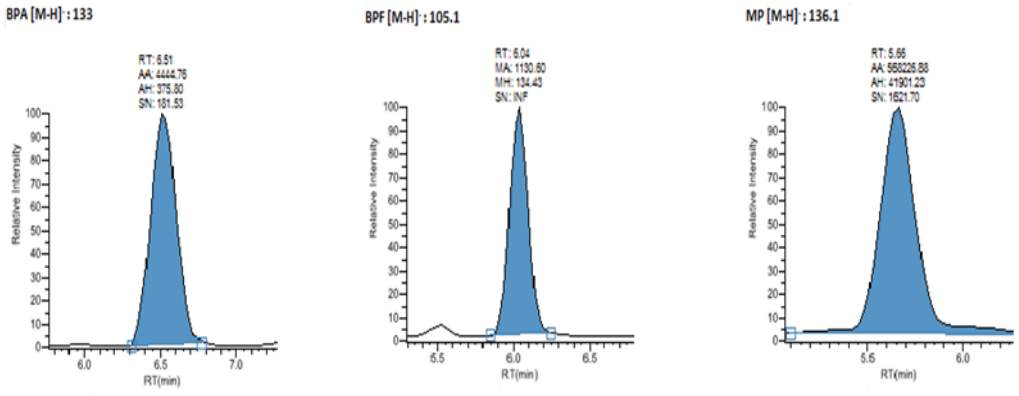


Figura 23. Cromatograma del ion diagnóstico del BPA (5.4 ng mL^{-1}), BPF (0.5 ng mL^{-1}) y MP (365 ng mL^{-1}) en una muestra de orina.

4.3.1. Conclusiones

- Se ha desarrollado una metodología rápida y sensible para la determinación de bisfenoles (BPA, BPF y BPS) y parabenos (MP, EP, PP y BP) en orina. El uso de una fuente APCI en el análisis LC-MS / MS reduce el efecto de la matriz y aumenta la sensibilidad del método para los bisfenoles, especialmente para el bisfenol A, obteniendo límites de detección mucho más bajos con fuente APCI.
- La preparación de la muestra es rápida y no requiere disolventes adicionales. La combinación de la preparación genérica de la muestra y la sensibilidad del análisis LC-MS / MS muestra su utilidad para futuros estudios de biomonitorización en orina humana.
- El método desarrollado se aplicó posteriormente a un estudio de biomonitorización en orina de madres lactantes en la Comunitat Valenciana.

4.3.4. ARTICULO 4. Analysis of four Parabens and Bisphenols A, F, S in urine, using Dilute and Shoot and liquid chromatography coupled to mass spectrometry

Talanta 202 (2019) 42–50

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Analysis of four parabens and bisphenols A, F, S in urine, using dilute and shoot and liquid chromatography coupled to mass spectrometry

Yovana Sanchis[a], Clara Coscollà[a], Vicent Yusà[a,b,*]

[a] Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020, Valencia, Spain
[b] Analytical Chemistry Department, Universit of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100, Burjassot, Spain

ARTICLE INFO

Keywords:
LC-MS/MS
Parabens
Bisphenols
Urine
Dilute and shoot

ABSTRACT

A new strategy for the analysis of 4 parabens (methylparaben, ethyl paraben, propyl paraben and butyl paraben) and 3 bisphenols (A, F and S) in human urine has been developed. Dilute and shoot and liquid chromatography coupled to mass spectrometry (LC-MS/MS) determination was employed. Procedural matched calibration, certified materials and spiked samples were used for validation. The obtained recoveries varied between 85 and 104% with a precision lower than 20% for all analytes. The LOQ was 0.20 ng mL for all analytes. Negative atmospheric pressure chemical ionization in the selected reaction monitoring mode was used for MS detection. The proposed method was successfully applied for the determination of these compounds in 15 human urine samples from lactating women from the Valencian region (Spain). Methyl paraben (MP) and Bisphenol A (BPA) presented high detection frequency (93% for MP and 80% for BPA), BPS and BPF presented a detection frequency of 33 and 26% respectively. Methyl paraben was detected in a concentration range of 0.2–2952 ng mL^{-1}, 0.2–12 ng mL^{-1} for bisphenol A, 0.5–39 ng mL^{-1} for BPF and 0.5–8.5 ng mL^{-1} for BPS.

1. Introduction

Bisphenols (BPs) are man-made chemicals used in various consumer products. Bisphenol A (BPA) is used in manufacturing of polycarbonate plastic and epoxy resins and is found in different products such us cans, dental sealants, thermal receipts, food packaging and personal care products (PCPs) [1]. Due to its estrogen activity and reproductive toxicity, BPA has a reduced use in the manufacture of feeding bottles in Europe since 2011 under Regulation 10/2011/EU [2] with a maximum specific migration limit (SML) of 0.6 mg kg Use of BPA for the manufacture of polycarbonate infant feeding bottles has been prohibited by the European Commission [3] and it is included in the REACH for its endocrine disrupting properties and for its toxicity [4–6]. Consequently, analogue compounds as Bisphenol S (BPS) and Bisphenol F (BPF) (Table 1) are used as a substitution for BPA in some of the consumer products. Although these substances are non-persistent chemicals and have short elimination half-lives in humans (6 h), their widespread use and potential endocrine disrupting properties have made them chemicals of concern [7].

Analytical methods for determination of bisphenols in urine have shown concern over contamination control mainly for bisphenol A. Contaminations of BPA may arise from laboratory accessories, reagent, extraction procedure, or the apparatus. Table 2 presents a review of recently published methods with special attention to the control of contamination. In general high quality solvents were used, glass material employed and procedural and reagent blank of sample analysed (Table 2).

After ingestion, BPA is metabolized and excreted, mainly in urine, with a half-life of < 6 h [10]. The metabolism includes glucuronidation or sulfatation, to increase its water solubility [16,]. The metabolism and elimination of BPF and BPS is less known, although it has been suggested that it is similar to BPA [17]. Urinary levels of free unconjugated bisphenols, are commonly used as biomarkers of recent exposure [13].

Parabens (alkyl esters of the *p*-hydroxybenzoic acid) are widely used as antimicrobial preservatives, especially in cosmetics, pharmaceuticals, and in food and beverages. Methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) are the most commonly used parabens (Table 1). The widespread use of parabens as preservatives arises from their low toxicity, broad inertness, worldwide regulatory acceptance and low cost. However, in recent years there has been a growing concern about human exposure to parabens and several in vitro and in vivo studies have reported estrogenic and antiandrogenic activity of these substances, in addition to decreased semen quality and testosterone levels in paraben-exposed male rodents [15]. Likewise, results have been conflicting, in vitro, animal and human studies have linked a range of parabens with changes in thyroid hormones. In

* Corresponding author. Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020, Valencia, Spain.
E-mail address: yusa_vic@gva.es (V. Yusà).

https://doi.org/10.1016/j.talanta.2019.04.048
Received 20 November 2018; Received in revised form 15 April 2019; Accepted 18 April 2019
Available online 24 April 2019

Table 1
Elemental composition, monitored ion mass and fragment ions.

Compound	CAS-number	Elemental composition	Diagnostic ion	Transitions (m/z)	Colision Energy(Ev)	Tube lens offset voltage (v)	RT Retention time (min)
Bisphenol A (BPA)	80-05-7	$C_{15}H_{16}O_2$	$[M-H]^-$	227.1/133.0[e]	−25	−78	
				227.1/212.0[f]	−22	−72	6.3
Bisphenol S (BPS)	80-09-1	$C_{12}H_{10}O_4$ S	$[M-H]^-$	249.1/92.1[e]	−25	−78	5.0
				249.1/108.0[f]	−25		
Bisphenol F (BPF)	620-92-8	$C_{13}H_{12}O_2$	$[M-H]^-$	199.0/105.1[e]	−25	−72	5.8
				199.0/92.9	−25		
Methylparaben (MP)	99-76-3	$C_8H_8O_3$	$[M-H]^-$	151.0/136.1[e]	−31	−78	5.34
				151.1//92.1[f]	−22	−72	
Ethylparaben (EP)	120-47-8	$C_9H_{10}O_3$	$[M-H]^-$	165.1/136.0[e]	−19	−72	6.0
				165.1/92.1[f]	−27	−51	
Propylparaben (PP)	94-13-3	$C_{10}H_{12}O_3$	$[M-H]^-$	179.1/136.1[e]	−16	−51	6.4
				179.1/92.0[f]	−22	−77	
Butylparaben (BP)	94-26-8	$C_{11}H_{14}O_3$	$[M-H]^-$	193.1/136.1[e]	−19	−77	6.6
				193.1/92.0[f]	−28	−78	
D_{16}-BPA[a]		$C_{15}D_{16}O_2$	$[M-H]^-$	241.2/141.	−20	−78	6.3
D_{10}-BPF[b]		$C_{13}H_2D_{10}O_2$	$[M-H]^-$	209.0/97.0	−22	−77	5.8
D_8-BPS[c]		$C_{12}H_2D_8O_4S$	$[M-H]^-$	257.0/112.0	−30	−51	5.0
$_{13}C_6$-BP[d]		$C^{13}_{13}C_6H_{14}O_3$	$[M-H]^-$	199.1/98.0	−28	−78	6.6
D_5- EP[e]		$C_9H_5D_5$	$[M-H]^-$	170.1/92.1	−31	−51	6.0

[e]SRM trasition used for quantification.
[f]SRM transition used for confirmation.
[a] Internal standard used for BPA.
[b] Internal standard used for BPF.
[c] Internal standard used for BPS.
[d] Internal standard used for BP.
[e] Internal standard used for MP,EP and PP.

addition, subclinical maternal thyroid dysfunction has been associated with low birth weight, low Apgar scores, and neurological disabilities [18].

Exposure to parabens may occur through ingestion, inhalation and dermal absorption. These compounds may conjugate to β-D-glucuronide and sulfate, which reduces their bioactivity and facilitates urinary excretion [15]. Generally, parabens are rapidly absorbed, metabolized, and excreted in urine from the body. Following excretion, the parent compounds can be measured in urine and have been shown to be valid biomarkers of exposure [19]. Sakhi et al. [7], confirm that these compounds are non-persistent chemicals and have short elimination half-life in humans, normally ranging from 1 to 7 h.

Human exposure to bisphenols and parabens is often monitored in urine samples, Table 2 summarizes some of the analytical procedures previously developed for the determination of parabens and Bisphenols in human urine. Although there are different methods for the determination of both Parabens and bisphenols using LC-MS/MS (Table 2) all of them require a sample preparation step that includes high volumes of solvents, more time and more possibility of BPA contamination.

In the present study we have developed a new analytical strategy based on a generic methodology, dilute and shoot and LC-APCI-MS/MS for a total determination of Bisphenols (A, F, S) and PBs (methyl-, ethyl-, propyl-, butylparaben) in human urine samples.

To our knowledge, there has been no previously reported work on the analysis of these.compounds in human urine using this fast and generic methodology. The proposed method was validated and applied in 15 urine samples from lactating mothers from the Valencian region (Spain).

2. Experimental

2.1. Chemicals and reagents

Methanol LC-MS grade was supplied by VWR Prolabo (Barcelona, Spain). Ultra-pure water produced with a Milli-Q Gradient system (Millipore, Bedford, USA) was used. Certified commercial standards were of high purity. BPA, BPF and BPS were purchased from Dr.Ehrenstorfer (Ausburg, Germany), MP, EP, PP and BP were supplied by Sigma-Aldrich(Barcelona, Spain), BPA-d16 was from Chromlab (Barcelona, Spain),D_{10}-BPF was purchased by TRC Canada laboratories, D_8-BPS and $^{13}C_6$-BP was supplied by Cambridge Isotope Laboratories (Massachusetts, USA) and D_5-EP was purchased from Santacruz Biotechnology (Heidelberg, Germany), Bisphenol S Monosulfate Disodium Salt, Bis(4- hydroxyphenyl) Sulfone O-β-D-Glucuronide Sodium Salt, Bisphenol F Mono- β-D-Glucuronide, Bisphenol F Monosulfate sodium Salt, Bisphenol A Monosulfate Sodium Salt, Bisphenol A β-D-Glucuronide were supplied by Toronto research Chemicals (Canada).

Reference material NIST 3673 Organic contaminants in non-smokers' urine supplied by Sigma Aldrich were stored in the dark at −20 °C.

For calibration solutions, each individual standard was weighed and dissolved in methanol to obtain a stock solution of approximately 200,000 ng mL^{-1}. The stock solutions were stored in the dark at −20 °C for less than 6 months. Intermediate standard solutions containing 1000, 100,000 and 10,000 ng mL^{-1} were prepared by diluting the individual stock solutions with methanol:water (20:80, v/v) and used for the procedural-match calibration curve and the spiked samples for recovery calculations. The intermediate solutions were stored in the dark at 4 °C for less than 2 months.

A mix solution containing the internal standards (Table 1) 1000 ng mL^{-1} was prepared by diluting individual standard solutions in methanol:water (20:80,v/v). This solution was stored in the dark at 4 °C for less than 2 months.

Calibration curves were constructed in two ranges 0.2–10 ng mL^{-1} and 10–300 ng mL^{-1}.

2.2. Sampling collection

The 15 samples of human urine analysed in this study are part of a biomonitoring project.

Urine sample collection from the women was obtained between 2 and 8 weeks after birth, during 2015 Mothers ranged from 32 to 39 years, who signed the consent and agreed to participate, received a Sampling protocol were approved by the Scientific Ethics Committee of

Table 2
Review of last analytical methodologies for Parabens and BPA, BPF, BPS.

Reference	Compounds	Sample volum (µL)	Sample preparation	Equipment	Ionization source	Calibration	LOD (ng/mL)	LOQ (ng/mL)	Detected Concentrations (ng/mL)	Control contamination
[8]	BPA, BPB, BPS, BPAF,BPF, MMP, MEP, MiBP, MCHP, MBP, MBzP, MEHP, MEOHP, MEHHP, MECPP, MOP, MCPP, MNP, MDP	50	Enzymatic hydrolysis +400 µL of formic acid 0.5% SPE-LC-MS/MS (Strata X 20 × 2.0 mm, 25 µm phenomenex)	LC-QTRAP-MS/MS with Synergi MAX-RP 150 × 3.00 mm, 4 µm	ESI (−)	Matrix matched calibration (synthetic urine)	BPA 0.10 BPS 0.067BPB 0.26 BPF 0.39	–	BPA [1.7–44.8] DF (%) 100 BPS [< 0.09–10] DF (%) 10 BPF [< 0.09–10] DF (%) 74	Bckaground contamination: Procedural of method blanks (n = 4) every batch. - glass material
[9]	BPA and derivates	300	Liquid-liquid extraction (with 10M ammonium formate)	UPLC system Acquity H Class, coupled to a Xevo TQ-S triple quadrupole mass spectrometer, UPLC column was an ACQUITY CSH C18 (1.7 µm particle size; 2.1 × 100 mm)	ESI (−)	Matrix matched calibration	–	0.5	BPA [ND-1.38]	- glassware, teflon seals - highquality solvent and reagent - Selectivity of the method was assessed by analyzing response from blank urine
[10]	MP,EP,PP,BP	200	Microextraction by packed sorbent	UPLC-MS/MS with Kinetex C18 column (100 mm × 2.1 mm × 1.7 µm)	ESI (−)	External calibration	–	0.5	MP [< LOQ-33.16] DF 50% EP [< LOQ-0.42] DF 37% PP [< LOQ-0.63] DF 97% BP[< LOQ-2.65] DF 57%	–
[11]	BPA, BPS, BPF, BPAF	2000	Enzymatic hydrolysis + liquid extraction with athylacetate	UHPLC-MS/MS Acquity BEH C18 column (2.1 mm × 100 mm; 1.7 µm;	ESI (−)	External calibration	BPS 0.010 BPF 0.10 BPA 0.09 BPAF 0.008	BPS 0.032 BPF 0.31 BPA 0.27 BPAF 0.024	BPA[ND-4.38] DF 70% BPS [ND-2.51] DF 40% BPF[ND-1.36] DF < 30%	- each step in the chemical analysis procedure were screened to confirm the absence of BPs. - solvents were proven to be free of BPs. - Only glassware baked for 4 h at 400 C in a muffle was used
[12]	BPA, BPS, BAP, BPP, BPF, BPAF, BPZ), 7 parabens (methyl-, ethyl-, propyl-, butyl-, benzyl-paraben, methyl-protocatechuic acid, and ethyl-protocatechuic acid), 5 benzophenones (benzophenone-1, benzophenone-2, benzophenone-3, benzophenone-8, and 4-hydroxybenzophenone), and two antimicrobials (triclosan and triclocarban)	5000	Enzymatic hydrolysis + air-assisted liquid-liquid microextraction	LC-MS/MS with Atlantis T3 dC18 column (75 mm × 2.1 mm i.d. and 3.0 µm	ESI (−)	matrix-matched calibration	BPF 0.25	BPS 0.07 BPF 0.25 BPA 0.1 MP 0.05 EP 0.05 PP 0.1 BP 0.1	BPA [< LOQ-19.6 DF 96% BPS[< LOQ-2.27] DF 14% BPF[< LOQ-4.68] DF 24% MP [2.29–1118] DF 100% EP[< LOQ-33.16] DF 78% PP[< LOQ-78.4] DF 84%	–

(continued on next page)

Table 2 (*continued*)

Reference	Compounds	Sample volum (µL)	Sample preparation	Equipment	Ionization source	Calibration	LOD (ng/mL)	LOQ (ng/mL)	Detected Concentrations (ng/mL)	Control contamination
									BP[< LOQ-11.13] DF 40%	
[13]	MP,EP,PP,BP, BPA, triclosan and benzophenone	300	Enzymatic hydrolysis + frozen overnight to precipitate cryophobic proteins	LC-MS/MS with 2 columns:RAM (Restricted Access Material) phase (LiChrospher® RP-8 ADS (25 µm) 25 mm × 4 mm RAM from Merck, Darmstadt, Germany), chromatographic separation was realized on a reversed phase C18 column (Atlantis dC18 30 mm × 150 mm; 3 µm, Waters, Ireland).	ESI (−)	Calibration standards (prepared in water)	–	–	MP[< LOQ-3230] DF 97% EP [< LOQ-238] DF 82% BPA[< LOQ-59.6] DF 95%	–
[14]	Bisphenols (BPA, BPS,BPF,BPP,BPZ,BPAP), benzophenones and parabens (MP,EP,PP,BP,BzP)	1000	Enzymatic hydrolysis + liquid-liquid extraction	UHPLC-TQMS with Scientific Betasil C18 column (2.1 mm 100 mm,3 µm)	ESI (−)	External calibration	BPS 0.2 BPA 0.2 MP 0.05 EP 0.05 PP 0.1 BP 0.1	–	BPA [8.27–20.6] BPS[0.15–1.60] The median concentrations of BPA [12.09], MeP [20.14], EtP [1.91]and PrP [1.34]	- pure - blank solutions every ten samples to monitor instrument background - The procedural blank solutions
[15]	MP,EP,PP,BP, benzophenones and BPA	–	Enzymatic hydrolysis + dispersive liquid–liquid microextraction (DLLME)	UPLC-MS/MS with C18 (50 mm × 2.1 mm I.D., 1.7 µm particle size) from Waters	ESI (−)	Matrix matched calibration	–	0.5	BPA [< LOQ-8.1]DF 70.6% MP [median concentration 34.9]DF 94.1% EP median concentration 1.8]DF 67.6% PP[median concentration 3.9]DF 70.6% BP[median concentration < 0.2]DF 38.4%	-Urine samples were collected in polypropylene containers free of BPA
[This one]	MP, EP,PP,BP, BPA,BPF,BPS	500	Enzymatic hydrolysis + Dilution	LC-MS/MS with C18(150 mm × 2 mm I.D., 5 µm particle size) from Phenomenex	APCI (−)	Calibration with procedural blank	BPA 0.066 BPS 0.066 BPF 0.066 MP 0.066 EP 0.066 PP 0.066 BP 0.066	0.2	BPA < LOQ-12 BPS < LOQ-8.8 BPF < LOQ-39 MP < LOQ-2952 EP < LOQ-9 PP < LOQ-4.7 BP < LOQ-0.5	- Only glassware baked for 4 h at 400 C in a muffle was used - highquality solvent and reagent - Selectivity of the method was assessed by analyzing response from blank urine

the Valencian Research Centre for Public Health (FISABIO) of the Valencian Government (Dirección General de Salud Pública, DGSP) and the Biomedical Scientific Ethics Committee of the University and Polytechnic Hospital La Fe.

Samples and data from donors included in this study were managed by the IBSP-CV BioBank (PT13/0010/0064), integrated in the Spanish National Biobanks Network and in the Valencian Biobanking Network and they were processed following standard operating procedures with the approval of the Ethical and Scientific Committees. Samples were stored until analysis at −80 °C.

2.3. Sample preparation

Urine samples were stored in 250 mL glass containers at −80 °C. Before analysis all samples were equilibrated to room temperature and homogenized. Aliquots of 500 µL urine were transferred into 10 mL glass centrifuge tube and 170 µL of ultrapure water was added. Each sample was spiked with 200 µL 1M ammonium acetate buffer at Ph 5.0, 25 µL of internal standard solution and 10 µL of β-glucuronidase/arylsulfatase solution (≥100,000 U/mL) for the hydrolysis of conjugated species. After incubation in the oven at 37 °C during 90 min, all samples were centrifugated at 4500 rpm during 10 min 400 µL of supernatant was transferred into glass vial and ultra-centrifugate at 13,000 rpm during 10 min. After, 250 µL were loaded into a conic vial with a microfilter insert. A volume of 20 µL was injected into the LC-MS/MS system.

2.4. HPLC-MS/MS analysis and ion source optimization

The HPLC–MS system consists on a Finnigan Surveyor Autosampler, LC Pump and TSQ Quantum Ultra detector (San José, CA, USA). Separations were obtained with Luna C18(2) column (150 mm × 2.00 mm ID, particle size 5 µm) from Phenomenex (Madrid, Spain). The flow rate used was 300 µL min^{-1} and the injection volume was 20 µL. Mobile phase consisted of (A) water and (B) methanol. The analysis started with 95% mobile phase A. Then, solvent A decreased to 0% in 7 min. The initial conditions were restored in 1 min, followed by a re-equilibration time of 2 min. Total run time was 10 min. Data acquisition was performed by the Thermo Scientific Trace Finder ™ 3.2 software.

Mass analysis was performed on the TSQ Quantum Ultra Detector analyzer equipped with an APCI source. The ion source settings were optimized by design of experiments: vaporization temperature 350 °C, capillary temperature 250 °C, discharge current 4 µA, sheath gas pressure 45 psi, auxiliary gas flow 4 arbitrary units working at collision gas pressure was 1.5 mTorr. Tube lens offset voltages were optimized for each compound using the automated optimization procedure in the syringe infusion mode provided by the manufacturer. Negative APCI mode and selected reaction monitoring (SRM) mode were used for all the compounds. Table 1 shows the monitored transitions for each compound.

The ion source was optimized with a central composite design study. The model was validated using a regression analysis of variance (ANOVA). MINITAB software was used in order to selection the maximum response of the seven compounds depending on the factor settings. This was done by using the “response optimizer”.

As there were multiple responses (one for each analyte), and as the response surfaces are different for each compound, it is necessary to find a factor setting that maximized the desirability for each response simultaneously.

2.5. Matrix effect

The matrix effect (ME) was evaluated according with the approach described previously [20,21]. The matrix effect was studied for all analytes and over the whole range of the calibration curve, using the formula:

$$\%\text{Matrix Effect} = (\text{slope MM} - \text{slope MS})/\text{slope MSx100}$$

where MM is the matrix-matched (addition of standars to a pool of human urine) and MS is the standards (addition of standards to water).

Each peak area was corrected with the area of its internal standard.

Negative values of matrix effects means suppression of the signal, and positive values enhancement. The values were categorized into three groups: (i) no matrix effect < ± 20%, (ii) medium > ± 20 and < ± 50%, and (iii) strong > ± 50% [22].

2.6. Control of contamination

The first step was the identification of the potential contamination sources of the method. Three points of the analytical method were susceptible to contamination: reagents, glassware and the use of microfilters before injection. Contamination from solvents and reagents during the analysis was monitored through the area response of reagent blank, procedural blank and mobile phase injections and the same for evaluate microfilters contamination. According with the guidance for bioanalytical method validation of the FDA [23], the blank value should not be higher than 20% of the residual level corresponding to the LOQ. On the other hand, the SANTE/11813/2017 [24] establishes a 30%. In the present work, no contamination was considered if the area of each compound in the control (reagent blank, procedural blank or mobile phase) was minor than 20% of the area for LOQ, following the FDA criteria.

In order to measure the accuracy of the entire experimental method, the analysis of certified material (NIST 3673) for BPA was done.

Glass material baked for 4 h at 400 °C in a muffle was used during sample collection and sample preparation and only high-quality solvent and reagent were used throughout the study.

2.7. Enzymatic hydrolysis, method validation and quality control

In order to evaluate the β-glucuronidase/arylsulfatase efficiency for the hydrolysis of conjugated species, a study about the enzimatic process was performed. A pool of blank human urine was spiked with the standards (Bisphenol S Monosulfate Disodium Salt, Bis(4- hydroxyphenyl) Sulfone O-β-D-Glucuronide Sodium Salt, Bisphenol F Mono-β-D-Glucuronide, Bisphenol F Monosulfate sodium Salt, Bisphenol A Monosulfate Sodium Salt, Bisphenol A β-D-Glucuronide) at two levels of concentration 10 ng ml^{-1} and 200 ng ml^{-1} (as free metabolites). Three replicates of each level were analysed. The incubation was carried out as indicated in section 2.3.

The method was validated according with SANTE/11813/2017 [24]. The linearity of the method was evaluated spiking water in two ranges of concentrations (from 0,2–10 ng mL^{-1} and from 10 to 300 ng mL^{-1}). Six points calibration curves were obtained with a 1/x weighting by plotting the quotients of peak areas of each analyte and the peak areas of the specific internal standard as a function of the concentration. Mandel's fitting test and $R^2 > 0.99$ were used in order to confirm the lineal model. The accuracy of the method was calculated as recoveries, and was carried out using a spiked human urine sample (pool of urine) at four levels of concentrations (0,2, 10, 100 and 300 ng mL^{-1}). Precision was assessed as coefficient of variation (%) over 5 days.

The limit of quantification (LOQ) was established as the lowest concentration validated with satisfactory recovery and precision (recoveries within 70–120%, repeatability RSD < 20%) [24].

In each analytical batch, various quality control samples (QC) were included. The QC were prepared by spiking human urine (pool of urine) at LOQ level and intermediate level of the calibration curves, A reagent blank and a blank of urine was included in each batch to test for contamination.

2.8. Quantification and confirmation criteria

For quantification, calibration curves with water in the two ranges of concentration described in 2.5 were used. The quantification was performed with the area corresponding to the most abundant fragment ion of each analyte. Isotopically-labelled internal standards were used (Table 1).

Compound identification and confirmation was based on SANTE/11813/2017 [24]: i) 2 products ions, ii) a tolerance of ± 0.1 min for Relative Retention Time and iii) Ion Ratio (IR < 30%) between precursor ion in the sample respect to the quality control.

3. Results and discussion

3.1. Chromatographic separation and ionisation mode

To obtain a good chromatographic separation different columns and mobile phases were tested. In the literature, several modifiers are used for determination of parabens and phenols in urine [8,13]. After multiple experiments, a mobile phase composed of water (a) and methanol (B) was selected as optimum. The use of this mobile phase produced the best response for the parent and product ions. Different assays which used mobile phases containing acetic acid produced low signal in the bisphenols (A, F, S) probably this fact is due to ionisation inhibition. For separation of individual compounds, we tested the following set of columns: C18 column (Symmetry, 2.1 × 150 mm, 5 µm) from Waters, Kinetex (byphenyl, 2,1 × 100 mm, 3 µm) from Phenomenex and Luna C18(2) column (150 mm × 2.00 mm ID, particle size 5 µm) from Phenomenex. The best chromatographic separation without acids in the eluents was achieved on Phenomenex and Luna C18(2) column (150 mm × 2.00 mm ID, particle size 5 µm).

3.2. Optimization the ion source

Electrospray ionization (ESI) is the most commonly technique used on the analysis of bisphenols and parabens in urine (Table 2). Other authors like H. Ayala and Y.Sanchis [25,26], shows the use of APCI for these compounds in food packaging. In our study a mix of 50 ng mL^{-1} was injected, six times for ESI(−) and APCI (−), obtaining ten times major signal for BPA and BPS in APCI(−) source, the rest of compounds (MP, EP, PP, BP and BPF) present similar signal response in both sources. Consequently, APCI (−) was selected as the ionization source.

In order to optimize the ion source efficiency a statistical design of experiments (DoE) have been developed. In view of the literature [27,28], the main factors affecting the APCI ion source are sheath gas pressure (SGP), capillary temperature (CT), auxiliary gas flow (AG), discharge current (DC), and vaporization temperature (VT). A central composite design (CCD) [29,30] was used. The design consisted of a two-level full factorial design 2^5, 8 cube points, 8 axial point and 10 central points in cube with 30 runs. The values corresponding to every factor in each determination and the responses for each analyte are shown in Supplementary information (Table SI-1).

It must be noticed that the desirability is 0.0 for the lowest values obtained in the CCD; and it increases as response values increase; being 1.0 for the highest response obtained in the experiments. The optimized responses had a global desirability of 0.644 and factor settings established were: VT 400 °C, CT 250 °C, DC 4 µA, SGP 45 psi and AG 4 arbitrary units.

3.3. Matrix effect and analytical performance of the method

In the study of the enzymatic hydrolysis, efficiencies higher than 90% were obtained both for glucuronides and sulfates of the three species (see Table 3).

To know the matrix effect (ion suppression or the signal enhancement) an extensive study explained in the point 2.5 was developed. Calibration curves for matrix effect are shown in supplementary data (Figs. SI-1 to SI-7 and Table SI-2). Both parabens and bisphenols presented ME lower than 20%, which, following the criteria described in 2.5, indicated no matrix effect.

This can be explained because sample dilution was employed and is a good option to avoid matrix effect. The APCI source is usually considered to be less affected by the matrix effects due to its different ionization manner in this case the ionization takes place in the gas phase [28]. As can be seen in Table 2 most of the methods for the determination of both parabens and bisphenols in urine employed ESI source matrix matched calibration to correct matrix effect.

Taking into account the absence of matrix effect, procedural water (same treatment that urine samples) was used for the calibration curves. Synthetic urine can also be used for calibration curves, because their slopes present no significant differences with those of water (data not shown).

The performance parameters of the method are shown in Table 3. Good linearity between 0.2 and 10 ng mL^{-1} and from 10 to 300 ng mL^{-1} was obtained with $R^2 > 0.99$. This range covers the compound levels found in field samples described in the literature [7,10,11,13,19].

Mean recoveries ranged from 85% (BPF and BP at lowest level) to 104% (BPA at lowest level) at four levels of concentration with coefficients of variation below 15%. Moreover, the experimental LOQ quantified with adequate precision, accuracy and identification was 0.2 ng mL^{-1} for all compounds and is comparable to those previously reported in the literature (Table 2). The intra and inter day precision of the method was assured by the calculated RSDs, which were < 20% in all cases. LOD was calculated as LOQ/3. LOD was 0.066 ng $mL^{-1}$for all compounds. This LOD is in the same order than the review literature (Table 2).

A chromatogram of the compounds from a human urine spiked at limit of quantification is presented in Fig. 1.

Taking into account the levels of these compounds in the general population, this method is useful for biomonitoring studies.

3.4. Control of contamination

No significant contamination was detected. Fig. 2 shows as an example BPA areas in reagent blank, mobile phase and LOQ for a batch. In all runs the BPA area for reagent blank and mobile phase were lower than 20% [23] of that of the LOQ. Likewise, in order to measure the possible contamination from BPA, reference material (NIST 3673) was injected in each batch and its average recovery was 90%.

3.5. Analysis of field samples

The developed analytical strategy was applied to 15 field human urine samples. The analysis allowed the identification and quantification of parabens and bisphenols in their total condition, as can be seen in Table 4. Fig. 3 shows the chromatogram of a field sample.

Methyl paraben (MP) and Bisphenol A (BPA) presented high detection frequency (93% for MP and 80% for BPA) Methyl paraben was detected in a concentration range of 0.2–2952 ng mL^{-1}, and 0.2–12 ng mL^{-1} for bisphenol A.Other studies presents high frequencies of detection (DF) for MP (Table 2) but this study presents high concentration levels comparable with previous two studies [12,13]. Concentrations of BPA are lower than [8,12–14] studies and higher than [10,11].

Four and six out of fifteen samples presents levels > LOQ, for BPF

Table 3
Validation Parameters and enzymatic hidrolysis efficiency.

Validation Parameters										
Compounds	**LOD(ng/ml)**	**LOQ(ng/ml)**	**0.2 ng ml^{-1}** recovery(%)	CV(%)	**10 ng ml^{-1}** recovery(%)	CV(%)	**100 ng ml^{-1}** recovery(%)	CV(%)	**300 ng ml^{-1}** recovery(%)	CV(%)
MP	0.06	0.2	99	15	102	13	96	6	99	7
EP	0.06	0.2	89	12	98	12	102	5	101	2
PP	0.06	0.2	102	11	87	8	99	9	107	7
BP	0.06	0.2	85	13	85	15	90	4	100	4
BPA	0.06	0.2	104	14	104	14	92	7	99	8
BPF	0.06	0.2	85	12	90	12	99	3	102	12
BPS	0.06	0.2	89	9	95	15	100	15	93	10

Enzymatic hidrolysis effeciency						
Levels (ngmL^{-1})	**BPA-glucururonide**	**BPA-sulfate**	**BPS-glucururonide**	**BPS-sulfate**	**BPF-glucururonide**	**BPF-sulfate**
10	99	93	90	90	96	90
200	97	94	100	95	100	95

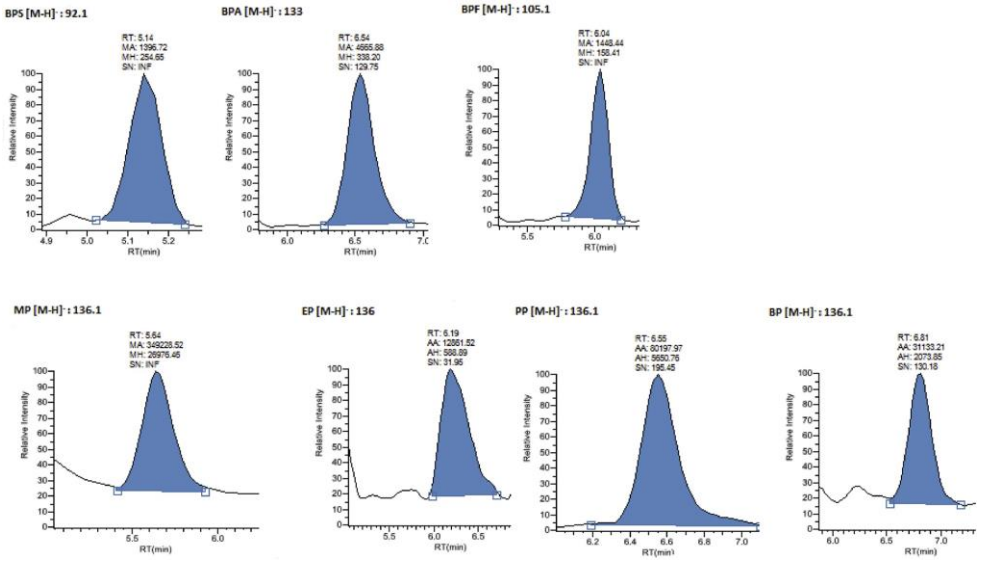


Fig. 1. Human urine spiked at limit of quantification (LOQ).

and BPS respectively with levels ranged from 0.5 to 39 ng mL^{-1},for BPF and from 0.5 to 8.5 ng mL^{-1}, from BPS. In a study from China BPS and BPF were detected in 22% and 30% of samples with geometric mean of 0.2 ng mL^{-1} [11]. Liao et al. [30] report urinary BPS from different countries with median concentrations of 0.9 ng mL^{-1} (Table 2).

EP, PP, and BP presented DF of 60, 40, 13% respectively, with concentrations ranging from < 0.2–9 ng mL^{-1} for EP, < 0.2–4.7 ng mL^{-1} for PP, and < 0.2–0.5 ng mL^{-1} for BP. Higher DF were founded in other studies (Table 2) for PP and BP but the number of samples analysed in this study are 15, this is not a biomonitoring study. However the detection and quantification limitis of the compounds shows the validity of the method for the analysis of total BPA, BPS, BPF and MP, EP, PP and BP in urine samples.

4. Conclusions

A rapid and sensitive methodology for the determination of bisphenols (BPA, BPF and BPS) and parabens (MP,EP, PP, and BP) has been developed. The use of an APCI source in the LC-MS/MS analysis reduces the matrix effect and increases the sensitivity of the method for bisphenols. To our knowledge this is the first report describing the determination of a mixture of parabens and bisphenols using an APCI source. Moreover, the sample preparation is fast and requires no additional solvents. The analytical method was successfully applied for the analysis of 15 human urine samples. The combination of the generic sample preparation and the LC-MS/MS analysis show its usefulness for future biomonitoring studies in human urine.

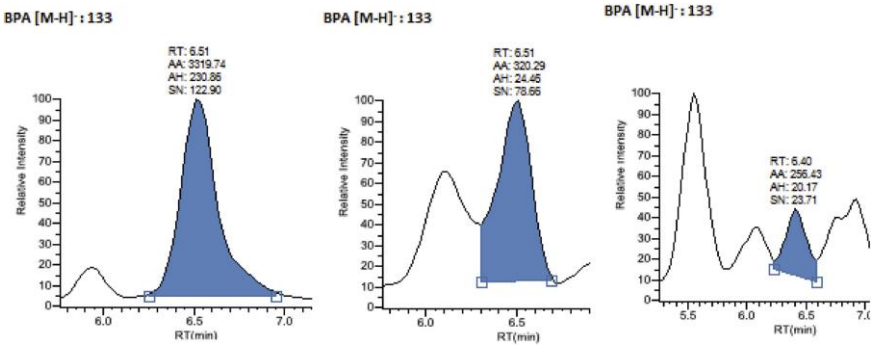


Fig. 2. BPA area in LOQ, reagent blank and mobile phase.

Table 4
Results (ng mL^{-1}) of compounds for monitorized samples.

SAMPLES															
Compounds	N = 1	N = 2	N = 3	N = 4	N = 5	N = 6	N = 7	N = 8	N = 9	N = 10	N = 11	N = 12	N = 13	N = 14	N = 15
MP	0.2	–	1230	2300	200	123	2952	452	2.8	25	256	365	856	754	852
EP	9	2.5	–	–	0.6	–	0.8	–	1.2	–	2.5	25	8	2.5	–
PP	2.7	4.7	–	0.8	–	–	0.5	–	–	–	0.6	–	–	1	–
BP	–	–	–	0.5	–	0.3	–	–	–	–	–	–	–	–	–
BPA	12	8	3	2.5	4	0.2	0.2	–	2.7	–	–	5.4	1.2	0.5	0.8
BPF	39	–	–	–	–	–	–	19	–	–	–	0.5	12	–	–
BPS	8.5	0.5	–	–	3.5	–	2.6	2.5	–	–	–	–	–	–	–

(–) = < LQ.

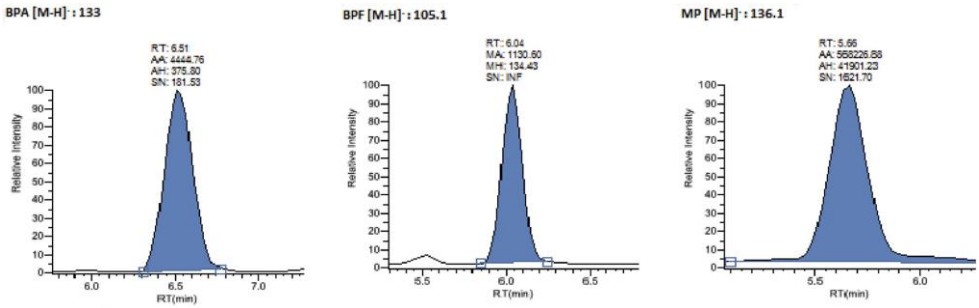


Fig. 3. Results of field sample: BPA, BPF and MP.

Acknowledgements

This study is part of the BETTERMILK project funded by the Generalitat Valenciana (GV/2015/008). The authors want to particularly acknowledge the donors and the IBSP-CV BioBank (PT/13/0010/0064) integrated in the Spanish National BioBank Network and in the Valencian Biobanking Network for their collaboration.

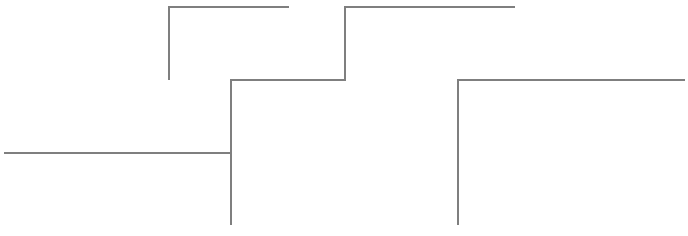
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2019.04.048.

References

[1] T. Geens, L. Bruckers, A. Covaci, G. Schoeters, T. Fierens, I. Sioen, G. Vanermen, W. Baeyens, B. Morrens, I. Loots, V. Nelen, B.N. de Bellevaux, N.V. Larebeke, E.D. Hond, Determinants of bisphenol A and phthalate metabolites in urine of Flemish adolescents, Environ. Res. 134 (2014) 110–117.
[2] Comission Regulation (EU), Nº 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Off.J.Eur. Union L12 (2011) 1.
[3] Comission directive 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles, (2011) http://eur-lex.europa.eu/legalcontent/EN/TXT/PDF/'uri=CELEX:32011L0008&from=EN , Accessed date: 9 January 2018.
[4] European Chemicals Agency ED/30/17, Inclusion of Substances of Very High Concern in the Candidate List for Eventual Inclusion in Annex XIV, (2017) Helsinki , Accessed date: 6 July 2017.
[5] European Chemicals AgencyED/01/2017, Inclusion of Substances of Very High Concern in the Candidate List for Eventual Inclusion in Annex XIV, (2017) Helsinki ,

Accessed date: 4 January 2017.
[6] European Chemicals Agency ED/01/2018, Inclusion of Substances of Very High Concern in the Candidate List for Eventual Inclusion in Annex XIV, (2018) Helsinki , Accessed date: 3 January 2018.
[7] A.K. Sakhi, A. Sabaredzovic, E. Papadopoulou, E. Cequier, C. Thomsen, Levels, variability and determinants of environmental phenols in pairs of Norwegian mothers and children, Environ. Int. 114 (2018) 242–251.
[8] A.L. Heffernan, K. Thompson, G. Eaglesham, S. Vijayasarathy, J.F. Mueller, P.D. Sly, et al., Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine, Talanta 151 (2016) 224–233.
[9] N. Venisse, C. Grignon, B. Brunet, S. Thévenot, A. Bacle, V. Migeot, et al., Reliable quantification of bisphenol A and its chlorinated derivatives in human urine using UPLC–MS/MS method, Talanta 125 (2014) 284–292.
[10] V. Cristína Jardim, L. de Paula Melo, D. Soares Domingues, M.E. Costa Queiroz, Determination of parabens in urine samples by microextraction using packed sorbent and ultra-performance liquid chromatography coupled to tandem mass spectrometry, J. Chromatogr. B 974 (2015) 35–41.
[11] Y. Yang, J. Guan, J. Yin, B. Shao, H. Li, Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China, Chemosphere 112 (2014) 481–486.
[12] B.A. Rocha, A.R.M. de Oliveira, F. Barbosa, A fast and simple air-assisted liquid-liquid microextraction procedure for the simultaneous determination of bisphenols, parabens, benzophenones, triclosan, and triclocarban in human urine by liquid chromatography-tandem mass spectrometry, Talanta 183 (2018) 94–101.
[13] R.K. Moos, J. Angerer, J. Wittsiepe, M. Wilhelm, T. Brüning, H.M. Koch, Rapid determination of nine parabens and seven other environmental phenols in urine samples of German children and adults, Int. J. Hyg Environ. Health 217 (8) (2014) 845–853.
[14] H. Zhao, J. Li, X. Ma, W. Huo, S. Xu, Z. Cai, Simultaneous determination of bisphenols, benzophenones and parabens in human urine by using UHPLC-TQMS, Chin. Chem. Lett. 29 (2018) 102–106.
[15] I. Jiménez-Díaz, F. Artacho-Cordón, F. Vela-Soria, H. Belhassen, J.P. Arrebola, M.F. Fernández, R. Ghali, A. Hedhili, N. Olea, Urinary levels of bisphenol A, benzophenones and parabens in Tunisian women: a pilot study, Sci. Total Environ. 562 (2016) 81–88.
[16] D. Mattison, N. Karyakina, M. Goodman, J.S. Lakind, Pharmaco-and toxicokinetics of selected exogenous and endogenous estrogens: a review of the data and identification of knowledge gaps (Review), Crit. Rev. Toxicol. 44 (2014) 696–724.
[17] L. Rochester, A. Bolden, S. and F. Bisphenol, A systematic review and comparison of the hormonal activity of bisphenol a substitutes, Environ. Health Perspect. 123 (2015) 643–650.
[18] A.M. Aker, L. Johns, T.F. McElrath, D.E. Cantonwine, B. Mukherjee, J.D. Meeker, Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: a repeated measures study, Environ. Int. 113 (2018) 341–349.
[19] J. Guo, C. Wu, D. Lu, S. Jiang, W. Liang, X. Chang, H. Xu, G. Wang, Z. Zhou, Urinary paraben concentrations and their associations with anthropometric measures of children aged 3 years, Environ. Pollut. 222 (2017) 307–314.
[20] L. Delma, N. Michlig, M. Gaggiotti, C. Guadalupe, R. Horacio, M. Repetti, Determination of glyphosate, AMPA and glyfosinate in dairy farm water from Argentina using a simplified UHPLC-MS/MS method, Sci. Total Environ. 645 (2018) 34–43.
[21] H. Kwon, S.J. Lehotay, L. Geis-Asteggiante, Variability of matrix effects in liquid and gas chromatography–mass spectrometry analysis of pesticide residues after QuEChERS sample preparation of different food crops, J. Chromatogr. A 1270 (2012) 235–245.
[22] Bożena Łozowicka, Ewa Rutkowska, Magdalena Jankowska, Influence of QuEChERS modifications on recovery and matrix effect during the multi-residue pesticide analysis in soil by GC/MS/MS and GC/ECD/NPD, Environ. Sci. Pollut. Res. Int. 24 (2017) 7124–7138.
[23] Guidance for INDUSTRY Bioanalytical Method Validation, U.S. Department of Health and a Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Veterinary Medicine (CVM), May 2011.
[24] SANTE/11813, European Commission Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed, (2017).
[25] H. Gallart-Ayala, O. Nunez, P. Lucci, Recent advances in LC-MS análisis of food-packaging contaminants, Trends Anal. Chem. 42 (2013) 99–124.
[26] Y. Sanchis, V. Yusà, C. Coscollà, Analytical strategies for organic food packaging contaminants, J. Chromatogr. A 1490 (2017) 22–46.
[27] Thermo Fischer Scientific TM. Training Course Manual Orbitrap Exactive™. European Training Institute.
[28] H. Mei, Y. Hsieh, C. Nardo, X. Xu, S. Wang, K. Ng, W. Korfmacher, Investigation of matrix effects in bioanalytical high-performance liquid chromatography/tandem mass spectrometric assays: application to drug discovery, Rapid Commun. Mass Spectrom. 17 (2003) 97–103.
[29] D.L. Massart, B.G.M. Vadeginste, C.M.C. Buydens, S. De Jong, P.J. Lewi, J. Smellers, V.R. Beke, Handbook of Chemometrics and Qualimetrics, Elsevier, Amsterdam, 1997.
[30] Chunyang Liao, Fang Liu, Husam Alomirah, Vu Duc Loi, Mustafa Ali Mohd, Hyo-Bang Moon, Haruhiko Nakata, Kurunthachalam Kannan, Bisphenol S in urine from the United States and seven asian countries: occurrence and human exposures, Environ. Sci. Technol. 46 (2012) 6860–6866.



Capítulo 4

4.4. Capítulo 4. Niveles de Bisfenoles (A, F y S) y Parabenos (MP, EP, PP, BP) en orinas de madres lactantes. Evaluación de la exposición y el Riesgo

4.4.1. Resumen

El método analítico desarrollado en el Capítulo 3 de la tesis, se aplicó a las muestras de orina procedentes de un estudio de biomonitorización en madres lactantes de la Comunidad Valenciana denominado "*Bettermilk*". Este capítulo presenta los niveles de bisfenoles y parabenos encontrados en la orina, y la correspondiente evaluación de la exposición y del riesgo.

Teniendo en cuenta la "toxicidad general" de los bisfenoles, se ha establecido una ingesta diaria tolerable temporal (t-IDT) de 4 µg kg $^{-1}$dia^{-1} para la exposición oral a BPA en población general (EFSA 2015). Respecto a los parabenos, la EFSA ha establecido una ingesta diaria aceptable (IDA) de 0-10 mg kg^{-1}día^{-1} para la suma de MP y EP (EFSA 2004a). Actualmente, no se ha establecido un valor de ingesta diaria aceptable para PP. El BPF y BPS, al igual que el BPA, ya se han incluido como sustancias prioritarias que se determinarán en los estudios de biomonitorización en humanos (HBM) (HBM4EU 2017).

En la literatura se describen varios estudios de biomonitorización para determinar los niveles de BPF y BPS en orina (Liao et al 2012; Yang et al 2014; Zhou et al 2014). El BPA y los parabenos se han investigado en la orina en una población de mujeres (Pollack et al. 2016; Buckley et al. 2016; Carita et al. 2013; Heffernan et al. 2016; Arbuckle et al. 2014; Sakhi et al. 2018; Jiménez-Díaz et al. 2016), pero solo se ha realizado un estudio en mujeres lactantes (Hines et al. 2015).

Lee et al. (2017) han estudiado los niveles de bisfenoles en muestras biológicas, como la leche materna. Varios estudios han descrito niveles de bisfenoles (media geométrica del BPA total: 0.29 ng/mL) y parabenos (MP promedio: 2.18 ng/mL) en leche materna (Dualde et al 2019; Schlumpf et al 2010). En consecuencia, los neonatos se exponen a bisfenoles y parabenos a través de la lactancia materna. Por esta razón, las madres lactantes son un grupo vulnerable de la población y es importante su estudio.

Los niveles de bisfenoles y parabenos en orina representan la exposición interna (dosis interna). Estos niveles pueden utilizarse en un contexto de evaluación del riesgo a través de la dosimetría directa, que compara los niveles determinados con los valores de

referencia basados en salud o valores guía, como los *"biomonitoring equivalents"* (BE) (Krishnan et al. 2010).

Otro enfoque para la evaluación del riesgo es mediante la dosimetría reversa, en la que se calcula la exposición externa a través de la ingesta diaria estimada (IDE) (µg ·kg^{-1} ·bw^{-1}· dia^{-1}), aplicando un modelo toxicocinético, y se compara con un valor de referencia como la ingesta diaria admisible (IDA) (Katsikantamia et al. 2019).

En este capítulo se han determinado los niveles de tres bisfenoles (BPA, BPS y BPF) y cuatro parabenos (MP, EP, PP y BP) en la orina de las madres lactantes valencianas que participan en el proyecto *Bettermilk*. Se han estudiado los factores que influyen en los niveles de bisfenoles y parabenos y finalmente, se ha realizado la evaluación del riesgo derivado de la exposición.

4.4.2. Discusión de resultados

a. Niveles de parabenos y bisfenoles en orina

Un total de 180 madres fueron seleccionadas para participar en el estudio. Se obtuvieron muestras de orina de 103 madres de 20 a 45 años, que representan una tasa de respuesta del 57 %. Las tablas 20 y 21 muestran las estadísticas descriptivas de los niveles de parabenos y bisfenoles referidas a las concentraciones de creatinina (µg·g^{-1} cret). El metilparabeno es la sustancia con mayor frecuencia de detección en las muestras (FD: 92%), seguida del bisfenol A que presenta una frecuencia de detección del 76% (figura 24). El BP, BPF y BPS, presentan valores de frecuencia de detección inferiores al 40% por lo que no se han considerado para los estudios de regresión.

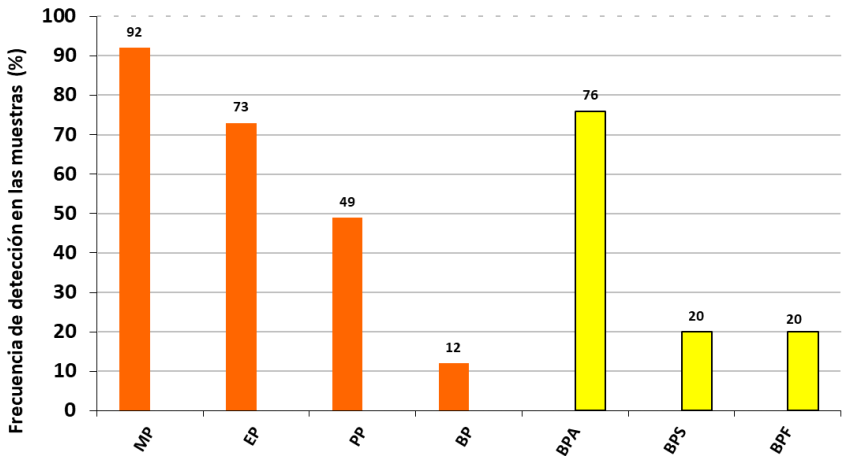


Figura 24. Frecuencia de detección en las muestras N=103.

Tabla 20. Niveles de parabenos en orina referidas a las concentraciones de creatinina (μg·g^{-1}cret) N=103.

	MP *(μg/gcret)*	**EP** *(μg/gcret)*	**PP** *(μg/gcret)*	**BP** *(μg/gcret)*
FD (%)	**92**	**73**	**49**	**12**
Mínimo	<LC	<LC	<LC	<LC
Percentil 25	2.5	0.18	0.02	0.006
Mediana	25.6	1	0.21	0.02
Media aritmética	171.5	8	6.1	0.1
Media geométrica	18	0.8	0.2	0.02
Percentil 75	154.8	2.6	1.6	0.08
Percentil 95	617	35.2	24.3	0.5
Máximo	4360	237	255	1.1
Desviación estándar	477	28	27	0.2

Tabla 21. Niveles de bisfenoles referidas a las concentraciones de creatinina (μg·g^{-1}cret) N=103

	BPA *(μg/gcret)*	**BPF** *(μg/gcret)*	**BPS** *(μg/gcret)*
FD (%)	76	20	20
Mínimo	<LC	<LC	<LC
Percentil 25	0.2	0.01	0.02
Mediana	1.3	0.04	0.08
Media aritmética	2.7	0.19	0.3
Media geométrica	0.94	0.04	0.06
Percentil 75	3.5	0.14	0.17
Percentil 95	9.7	1	0.6
Máximo	40	1.9	14.1
Desviación estándar	4.7	0.37	1.4

Distintos estudios han descrito sobre las concentraciones de determinados parabenos en la orina de adultos (Quiros-Alcalá et al. 2018) y mujeres (Sakhi et al. 2018; Jimenez-Díaz et al. 2016; Pollack et al. 2016) en Estados Unidos, China, Noruega y Túnez. Sin embargo, la información sobre las concentraciones de parabenos en la orina de las poblaciones más

vulnerables, como las mujeres embarazadas o en período de lactancia, es limitada. Hines et al. (2015) publicaron el único estudio para madres lactantes, en el que investigaron diferentes concentraciones de parabenos en su orina en el periodo de 2004 a 2005 en los Estados Unidos. Los cuatro parabenos (MP, EP, PP y BP) detectados en 34 madres lactantes por Hines et al. (2015) también se han detectado en nuestro estudio. En todos los casos los niveles encontrados en EE. UU. fueron más altos que en nuestro estudio.

Respecto a los bisfenoles, las concentraciones más altas de BPA (medias geométricas) se han descrito en mujeres estadounidenses (2,04 µg/g de creatinina) (Pollack A. et al 2016) y en mujeres embarazadas australianas (1,95 µg/g de creatinina) (Carita et al. 2013). En el presente estudio la media geométrica es de 0,94 µg/g de creatinina. Con respecto a la exposición de los bisfenoles en la orina de mujeres lactantes, Hines et al. (2015) es el único estudio en la literatura, y detectó concentraciones más elevadas en el percentil 75^{th} (4.1 ng mL^{-1}) que las encontradas en el presente estudio (2.9 ng mL^{-1}).

Estudios previos en todo el mundo, uno en Australia (Heffernan et al. 2016) y otro en Noruega (Sakhi et al. 2018), determinaron los niveles de BPS y BPF en la orina de mujeres. No se encontraron BPS ni BPF en la orina de mujeres embarazadas australianas (Heffernan et al. 2016). Sin embargo, Sakhi et al. (2018) informaron medias geométricas de 0,11 ng mL^{-1} y 0,08 ng mL^{-1} para BPS y BPF, en mujeres noruegas no embarazadas. Estos niveles son mayores que los encontrados en nuestro estudio.

b. Factores de influencia en los niveles de parabenos y Bisfenol A en orina.

Aplicando los modelos de regresión lineal simple, se investiga la asociación entre los niveles de los parabenos (MP, EP y PP) y bisfenol A (BPA) en orina, con las características y hábitos de la población estudiada. Resultando significativos en el modelo de regresión simple, el consumo de productos de la pesca, comida envasada, y el uso de perfumes (diariamente y varias veces por semana) con los niveles de MP. Para el PP, se detecta una relación significativa con el consumo de pasteles y el uso de cosméticos (cremas), diariamente o varias veces por semana y en el caso del BPA resultó significativo el consumo de cereales y legumbres, el uso de cremas alguna vez al mes y el uso de perfumes diariamente.

En los modelos de regresión lineal simple, no se observó asociación entre las variables edad del participante, "tener el primer embarazo", el IMC (índice de masa corporal) y la

concentración de BPA en orina. Philips et al. (2018) tampoco encontraron asociación entre la edad materna y las concentraciones de bisfenoles. Otros estudios han encontrado asociación entre el IMC materno, el tabaquismo, la edad y grado de educación bajo con niveles elevados de BPA (Callan et al 2013; Arbuckle et al 2014; Valvia et al 2015).

Considerando los resultados obtenidos del estudio de regresión lineal simple, se realizó el análisis multivariante (regresión lineal múltiple) con aquellas variables que presentan una asociación significativa en la regresión lineal simple. En el caso del MP resultaron significativos el consumo de productos de la pesca y el uso de perfumes varias veces por semana. El consumo de productos de la pesca, aunque es estadísticamente significativo, el impacto es muy bajo, ya que el coeficiente estimado para dicha variable es bajo (ver tabla 22).

Las mujeres que utilizan perfumes varias veces a la semana tienen una relación estadísticamente significativa con la concentración de MP, a mayor consumo de perfumes por semana mayor concentración de MP en orina.

Tabla 22. Resultados del modelo de la regresión lineal múltiple para los niveles log (MP) en orina.

Variable	Coeficiente estimado (95% CI)	Error standard	P-valor
Intersección	-25.5186 (-49.1037 - -1.9335)	11.6401	0.0347*
Productos de la pesca (g/mes)	0.0003 (0.00004 - 0.0006)	0.0004	0.0256*
Perfumes: varias veces por semana	2.6393 (0.7646 - 4.5139)	0.9252	0.0071*

*P-valor ≤ 0.05.

Respecto al EP, los resultados obtenidos en el estudio de regresión múltiple reflejan una relación estadísticamente significativa con el consumo de legumbres y cereales (tabla 23).

Tabla 23. Resultados del modelo de la regresión lineal múltiple para los niveles log (EP) en orina.

Variable	Coeficiente estimado (95% CI)	Error standard	P-valor
Intersección	4.7844 (2.1253 - 7.4434)	1.3386	0.0006*
Legumbres y cereales (g/mes)	0.0002 (0 – 0.0003)	0.0001	0.0239*

*P-valor ≤ 0.05.

En el caso del PP, ninguno de las variables significativas en el modelo de regresión lineal lo ha sido al aplicar el modelo de regresión múltiple. Jiménez-Díaz et al. (2016)

encontraron asociación, utilizando la prueba U de Mann-Whitney, entre la edad y los niveles urinarios del PP, probablemente debido al uso más frecuente de productos de cuidado personal en mujeres jóvenes en comparación con mujeres mayores. También encontraron que las mujeres que trabajan fuera del hogar mostraron niveles más altos del PP en la orina que las que trabajaban en casa, lo que sugiere un mayor uso de cosméticos y alimentos procesados en las mujeres que trabajan fuera del hogar. En nuestro caso no se ha encontrado asociación con ninguna de las variables.

Para el BPA, se obtuvo una relación significativa entre las madres que consumían fruta frente a las que no la consumían, sin embargo, el coeficiente estimado es muy bajo y, por tanto, aunque la variable sea significativa su contribución es irrelevante (tabla 24). El uso de perfumes diario presenta un coeficiente estimado elevado y positivo, lo que implica que las mujeres que utilizaban perfumes diariamente presentaban mayores niveles de bisfenol A en orina que las que no utilizaban perfumes. También resultó significativa la utilización de perfumes "alguna vez al mes", si bien únicamente cuatro madres dieron esta respuesta.

Tabla 24. Resultados del modelo de la regresión lineal múltiple para los niveles log (BPA) en orina

Variable	Coeficiente estimado (95% CI)	Error standard	P-valor
Intersección	-10.9894 (-19.9693 - -2.0095)	4.5179	0.017*
Frutas (g/mes)	$3.29 \cdot 10^{-5}$ ($3.79 \cdot 10^{-6}$ – 0.0001)	$1.46 \cdot 10^{-5}$	0.0272*
Perfumes: diario	1.0128 (0.2144 - 1.8111)	0.4017	0.0135*
Perfumes: alguna vez al mes	1.7798 (0.1042 - 3.4554)	0.843	0.0376*

*P-valor ≤ 0.05.

Tanto el uso de productos de cuidado personal (PCP) como el consumo de alimentos fueron los principales factores predictores de los niveles de bisfenoles y parabenos en el modelo de regresión lineal múltiple. El uso de PCP es una de las principales fuentes de exposición a los parabenos.

Al explorar los determinantes de la dieta, algunos grupos de alimentos se asociaron significativamente con los niveles de MP (pescado), EP (legumbres y cereales) y BPA (frutas) en orina. Sin embargo, otros estudios no han identificado un patrón claro con ninguno de los grupos de alimentos consumidos y los bisfenoles estudiados (Sakhi et al. 2018). Si bien algunos estudios han demostrado que el consumo de alimentos enlatados

es un importante predictor del BPA en orina, en el presente trabajo no hemos encontrado esta asociación (Sakhi et al. 2018; Philips et al. 2018). Se encontraron asociaciones positivas, aunque débiles, entre el BPA y el consumo de fruta. Se cree que la ruta principal de exposición al BPA es la dieta y se sugiere que las principales fuentes de alimentos son los productos enlatados como la carne, verduras o frutas enlatadas (Callan et al 2013). Desafortunadamente, en nuestro cuestionario no tenemos información sobre el porcentaje de fruta enlatada que consume nuestra población. Observamos concentraciones ligeramente más altas de MP y EP en madres lactantes que habían consumido productos de pesca y legumbres o cereales. Probablemente, esto se debe a que los parabenos se han usado principalmente como conservantes antimicrobianos en estos productos alimenticios (Sakhi et al 2018). Jiménez-Díaz et al. (2016) describieron que el consumo de cereales se asoció positivamente con los niveles urinarios de MP, probablemente debido a la migración de este compuesto de los envases de plástico antibacteriano. Sin embargo, Philips et al. (2018) encontraron concentraciones más bajas de BPS en mujeres embarazadas que habían comido una gran cantidad de granos de cereales, mientras que en mujeres con un alto consumo de pescado y mariscos se encontraron concentraciones más altas de BPA (Philips et al. 2018).

c. Evaluación de la exposición y riesgo

Se realizó una evaluación del riesgo para los compuestos que presentaron una FD > 40%, BPA, MP, EP y PP. La evaluación de riesgo se estimó en función de los valores de referencia disponibles para cada compuesto. En el caso del BPA se dispone del valor de BE de 2 mg l^{-1} (Krishnan et al. 2010), por lo que para la evaluación del riesgo en este caso se calcula el cociente de peligro que consiste en el cociente de la concentración correspondiente al percentil 95 y el valor de BE. El BPA tendría un cociente de peligro de 0.0049 < 1 (tabla 25).

Tabla 25. Ingestas diarias estimadas, índice de riesgo, valores de referencia y márgenes de seguridad

MP and EP					
$P95^a$ [mg/L]	$IDE_{optimista\ (MP+EP)}$ [mg/kg·dia]	$IDE_{pesimista}$ (MP+EP) [mg/kg·dia]	IDA [mg/kg·dia]	$HQ_{optimista}$	$HQ_{pesimista}$
0.486 (**MP**)	0.0108	0.0434	10	0.001	0.004
0.0205 (**EP**)					
PP					
$P95^a$ [mg/L]	$IDE_{optimista}$ [mg/kg·dia]	$NOAEL_{[mg/kg·dia]}$	**Margen de Seguridad**		
0.02	0.00042	6.5	15476		
BPA					
$P95^a$ [mg/L]	$BE_{[mg/L]}$	HQ			
0.0097	2	0.0049			

a: Percentil 95.

El panel de expertos de la EFSA (2004a) estableció una IDA de 0-10 mg /kg /día para la suma de MP y EP. Sin embargo, no se ha establecido una IDA para PP. En consecuencia, para el compuesto PP, se calculó un margen de seguridad según lo descrito por Boberg et al. (2010).

En el caso de MP y EP, la evaluación de riesgo se estimó utilizando los HQ como indicadores y se calcularon según la *Ecuación 1 y la 2* descritas en el apartado *3.6.2* (material y métodos). La suma de MP y EP tendría un valor de IDE pesimista de 0.04 mg/kg/día y optimista de 0.01 mg/kg/día. Conociendo que el valor de ingesta diaria admisible para el MP y EP es de hasta 10 mg/kg/día (EFSA 2004a), el HQ para la situación pesimista sería de 0.004 y para la optimista de 0.001. Ambos son <1 por lo que no representarían ningún riesgo. Para el PP el IDE tendría de valor pesimista de 0.00042 mg/kg/día y un NOAEL de 6.5 mg /kg/día. Según el estudio de Boberg et al. (2010), con estos valores el PP presentaría un margen de seguridad de 15476 (tabla 25). La EFSA establece que las sustancias con margen de seguridad superior a 10000 no implican riesgo (EFSA 2005d).

De manera similar a nuestro estudio, Sakhi et al. (2018) estimaron las ingestas diarias de diferentes fenoles ambientales y parabenos en madres e hijos concluyendo que ninguno de los participantes excedió la IDA para BPA, MP y EP según lo establecido por la EFSA. Callan et al. (2013) calculó la ingesta diaria media de BPA para una población de mujeres embarazadas de Australia, la ingesta estimada obtenida (rango 0.01-0.14 µg /kg·día) fue

de 3 órdenes de magnitud por debajo de la ingesta diaria tolerable europea para BPA y la dosis de referencia de US EPA de 50 µg / kg·día.

Respecto a los parabenos, en nuestro estudio, la suma de MP y EP obtenida en un escenario pesimista fue de 0.0434 mg/kg·día. Este valor es inferior a la IDA establecida por la EFSA (10 mg/kg·día) (EFSA, 2004).

Como se describe en la tabla 25, las ingestas diarias calculadas son seguras para las madres lactantes, ya que HQ es tres órdenes de magnitud inferior a 1 para todas las sustancias estudiadas.

4.4.3. Conclusiones

- Tres bisfenoles (BPA, BPF y BPS) y cuatro parabenos (MP, EP, PP y BP) fueron detectados en la orina de madres lactantes en la Comunitat Valenciana. Respecto a los bisfenoles, el BPA es la sustancia más detectada (FD = 76%) con concentraciones de hasta 40 µg/g de creatinina. Compuestos análogos al BPA, como son el BPF y BPS que vienen reemplazándolo, presentan bajas frecuencias de detección en la población estudiada (FD =20%). El MP y EP son los parabenos con mayor frecuencia de detección en orina obteniendo concentraciones medias de 18 µg/g y 0.8 µg/g respectivamente.
- En este estudio, se ha observado una asociación entre el uso de productos de cuidado personal y el consumo de alimentos con una mayor concentración a bisfenoles y parabenos. El uso de perfumes se asoció a mayores concentraciones de MP y BPA. Dos parabenos (MP y EP) y un bisfenol (BPA) mostraron una asociación con el consumo de algunos grupos de alimentos, como productos de pesca, legumbres, cereales y frutas.
- La exposición estimada ha resultado ser de tres órdenes de magnitud por debajo de los valores de referencia para la evaluación del riesgo, por tanto, se puede concluir que no hay riesgo para la población de madres estudiada.

4.4.4. Artículo 5. Biomonitoring of bisphenols A, F, S and parabens in urine of breastfeeding mothers: exposure and risk assessment. Science of Total Environment (En revision)

Biomonitoring of bisphenols A, F, S and parabens in urine of breastfeeding mothers: exposure and risk assessment

Yovana Sanchis [a,b], Clara Coscollà[a,b] , Francisca Corpas-Burgos[a], Máximo Vento[d] , María Gormaz[d] and Vicent Yusà[a,b,c] and Bettermilk Project[1*]

[a] Foundation for the Promotion of Health and Biomedical Research in the Valencian Region, FISABIO-Public Health, 21, Avenida Catalunya, 46020, Valencia, Spain.

[b] Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain.

[c] Analytical Chemistry Department, University of Valencia, Edifici Jeroni Muñoz, Dr. Moliner 50, 46100 Burjassot, Spain.

[d] Neonatal Division at the University and Polytechnic Hospital La Fe, Avenida Abril Martorell, 106, 46026 Valencia, Spain

Abstract

In the present study we use human biomonitoring to assess the internal exposure and the risk to four parabens and three bisphenols in 103 Spanish breastfeeding mothers participating in the BETTERMIILK project. Urinary methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP), presented detection frequencies ranging from 92 % (MP) to 12 % (BP), while Bisphenol A (BPA), Bisphenol F (BPF) and Bisphenol S (BPS) were detected in 76 % (BPA) and 20% (PBF,S) of the mothers. Average parabens concentrations (GM: geometric mean) ranging from 0.021 ng mL^{-1} (BP) to 1.245 ng mL^{-1} (MP), whereas bisphenols had GM concentrations from 0.042 ng mL^{-1} (BPF) to 0.381 ng mL^{-1} (BPA). For risk assessment, direct (BPA) and reverse dosimetry (Parabens) were used. Hazard quotients (HQ) < 1 were calculated for both parabens and bisphenols.

Sociodemographic characteristics, food consumption and personal care products usage (PCPs) patterns have been investigated as possible determinants of exposure. Regarding to PCPs, use of parfums was significantly associated with higher urinary levels of parabens and bisphenols. Regarding to food consumption, positive associations were found between BPA and fruit

consumption. Moreover, higher concentrations of MP and EP in breastfeeding mothers consuming fish fishing products and legumes/cereals were found.

Keywords: Biomonitoring, Breastfeeding mothers, parabens, phenols, Urine.

*Corresponding author. Tel.: +34 961925865; fax: +34 961925704.
E-mail addresses: yusa_vic@gva.es (V. Yusà)
[1] Bettermilk project: E. Torres, M. Cernada, P. Correcher, A. Nuñez, A. Parra and A. Ramón

1. Introduction

Bisphenols (BPs) and Parabens are man-made chemicals used in various consumer products. Bisphenol A (BPA) is used in manufacturing of polycarbonate plastic and epoxy resins and is found in different products like cans (food and drink), dental sealants, thermal receipts, food packaging and personal care products (PCPs). Due to its toxicity, BPA has been banned in manufacture of infant feeding bottles in Europe since 2011. Consequently, bisphenols analogues like Bisphenol S (BPS) and Bisphenol F (BPF) are used as a substitution for BPA in some of the consumer products (Chen et al. 2016). Parabens (alkyl esters of the p-hydroxybenzoic acid) are widely used as antimicrobial preservatives, especially in cosmetics, pharmaceuticals, and in food and beverages. Methylparaben (MP), ethylparaben (EP), propylparaben (PP) and butylparaben (BP) are the most commonly used parabens. The widespread use of parabens as preservatives arises from their low toxicity, broad inertness, worldwide regulatory acceptance and low cost (Jardim et al. 2015). Diet is considered an important source of bisphenols. Certain food groups such as canned food, fish, meat and poultry have been associated with bisphenol levels (Sanchis et al. 2017). Exposure to parabens may occur through ingestion, inhalation and dermal absorption (Darbre et al. 2008).

After oral ingestion, BPA suffers a metabolic processes, and is excreted mainly as BPA-glucuronide in urine with a half-life of less than 6 h. Bisphenols analogues (BPS and BPF) metabolism in urine is less known, but it seems to be similar to BPA (Rochester et al 2008). Parabens may conjugate to β-D-glucuronide and sulphate, which reduces their bioactivity and facilitates urinary excretion .Generally, parabens are rapidly absorbed, metabolized, and excreted in urine from the body. Following excretion, the parent compounds can be measured in urine and have been shown to be valid biomarkers of exposure (Guo et al., 2017). Sakhi et al. (2018) confirm that parabens have short elimination half-life in humans, normally between 1-7 hours. Although these substances, both bisphenols and parabens, are non-persistent chemicals

and have short elimination half-lives in humans, their widespread use and potential endocrine disrupting properties have made them chemicals of concern (Sakhi et al.,2018).

European plastic material regulation has established the migration limit of 0.05 mg of BPA per kg of food (EU, 2011) and has banned the use of BPA in baby bottles and prohibited the migration of BPA from varnishes or coatings applied to materials in contact with food for infants and children 0-3 years old (EU, 2018). Taking into account the "general toxicity" of bisphenols, only a temporary tolerable daily intake (t-TDI) of 4 µg kg $bw^{-1}day^{-1}$ has been established for oral exposure to BPA (EFSA, 2015). In the case of parabens, EU allows and regulates the use of parabens in cosmetic products, food and pharmaceuticals. In addition, EFSA has established an acceptable daily intake (ADI) of 0-10 mg kg $bw^{-1}day^{-1}$ for the sum of MP, EP acid esters and their sodium salts based on studies which showed non-observed-adverse-effects-levels (NOAELs) for both parabens. Until now it has not been established an acceptable daily intake for PP (EFSA, 2004).

Apart from BPA, BPF and BPS have been already included as prioritised substances to be determined in human biomonitoring studies (HBM) (HBM4EU, 2017). Several biomonitoring studies have been implemented in order to determine urinary levels of BPF and BPS (Liao et al 2012; Yang et al 2014; Zhou et al 2014). Although some HBM researches for bisphenols and parabens in urine have been performed in women population (Pollack et al., 2016; Buckley et al., 2016; Carita et al. 2013; Heffernan et al., 2016; Arbuckle et al., 2014; Sakhi et al., 2018; Jiménez-Díaz et al., 2016), only one has studied breastfeeding women (Hines et al. 2015). Lee et al. (2018) have studied the distribution of bisphenols in biological samples such as urine and human milk for pregnant women, before delivery, at delivery and 1 month after delivery. Levels of BPA in urine were 2.23 ng/mL and 0.51 ng/mL in human milk. Several studies have described levels of bisphenols (geometric mean of total BPA: 0.29 ng/mL) and parabens (mean MP: 2.18 ng/mL) in human milk (Dualde et al 2019;Schlumpf et al 2010). Consequently, neonates are exposed to bisphenols and parabens through breastfeeding, and the levels of these substances in human milk are determined for the exposure of the mothers. For this reason, lactating mothers is a vulnerable group of population, and it is important to be studied more in depth.

Human exposure to bisphenols and parabens can be estimated through both external and internal approach (Yusà et al. 2018). The internal exposure approach is based on internal dose measured through human biomonitoring, and these data can be used in a risk assessment context by both direct and reverse dosimetry.

For risk calculation using direct dosimentry we compare the internal dose of pollutants in biological fluids with health-based reference values or exposure guidance values such as Biomonitoring Equivalents (BE) (Krishnan et al., 2010). When guidance values are not available, reverse dosimetry can be used to assess external exposure using internal dose. Katsikantami et al. (2019) adopted this approach and calculated the Estimated Daily Intake (EDI) from urinary measurements of biomarkers. Once an EDI was estimated, risk assessment was performed calculating the hazard quotient (HQ) that is the ratio of EDI to Acceptable Daily Intake (ADI) of the substance. (Katsikantami et al. 2019; Yusà et al. (2018)

The aim of the present study was: i) to determine the levels of three bisphenols (BPA, BPS and BPF) and four parabens (MP, EP, PP and BP) in the urine of breastfeeding mothers living in Valencia (Spain) participating in the BETTERMILK; ii) to study the factors influencing the bisphenols and parabens levels; iii) to estimate the exposure and the risk assessment to bisphenols and parabens.

2. Material and methods

2.1. Study design and sample collection

A total of 180 breastfeeding women aged between 20 and 45 were recruited from June to November 2015 at the University and Polytechnic Hospital “La Fe” (Valencia, Spain) as a part of the Bettemilk project. Details of the study design were described previously (Yusà et al., 2017). 103 out of the 180 lactating women donated urine samples and were investigated.

Urine sample collection was obtained between 2 and 8 weeks after birth. Mothers who signed the consent and agreed to participate, received a questionnaire in the Hospital to complete at home and a kit (disposable gloves and a sterile 100 mL polypropylene bottle) with instructions on how to collect the first-spot morning urine sample. In addition, they were informed about which day they had to prepare the fulfilled questionnaire and the urine sample, and when researchers would collect both things at home.

Self-reporting questionnaires with detailed information on socio-demographic characteristics, lifestyles, cosmetic use and diet (food frequency consumption) were administered to the studied population. Food consumption frequency by groups was provided for filling and converted to semi-quantitative intakes, as explained in detail by Yusa et al (2017). 72-hour reminder questionnaire was also provided in a face-to-face interview, to mothers between 2 and 8 weeks after birth, in order to assess the recent exposure. Questions on the use of plastic food storage and consumption of canned food were included.

Samples and data from donors included in this study were managed by the IBSP-CV BioBank (PT13/0010/0064), integrated in the Spanish National Biobanks Network and in the Valencian Biobanking Network and they were processed following standard operating procedures with the approval of the Ethical and Scientific Committees. Samples were stored until analysis at -20ºC.

The study and sampling protocol were approved by the Scientific Ethics Committee of the Valencian Research Centre for Public Health (FISABIO) of the Valencian Government (Dirección General de Salud Pública, DGSP) and the Biomedical Scientific Ethics Committee of the University and Polytechnic Hospital La Fe. Before inclusion in the study, potential participants were informed about the aim and relevance of the investigation. All participants signed an Informed Consent approved by the Ethic Committee. To protect the participant´s privacy, all the collected personal data and biological samples were coded and used only for the research purposes.

2.2 Chemical analysis and Quality assurance/Quality control (QA/QC).

The details of the sample preparation, LC-MS/MS analysis, analytical and performance of the method were described in a previous paper published by Sanchis et al. (2019). Creatinine analysis were done according to the kinetic methodology based on the Jaffé alkaline picrate reaction (Larsen, 1972) using an automatic analyser (Linear Kroma, USA).

Samples were analysed under the quality system protocols following the ISO/IEC/EN 17025. Quality control samples to check the performance of the method were used in each batch, including reagent blanks, matrix blanks and spiked blank samples. The method was validated according to the SANTE /11813/2017 guideline (SANTE, 2017) defining the following performance criteria: recoveries within 70-120 %; repeatability RSD≤ 20%. The limit of quantification (LC) was defined as the lowest level of analyte that can be determined with acceptable recovery and precision. In order to assess the linearity, analysis of variance (ANOVA) Mandel's fitting test and a R^2 >0.99 was required.

Compound identification and confirmation were based on these criteria: (i) Two ions, one for quantification and another for confirmation. In case of use the internal standard only one ion was required; (ii) ion ratio similar to the standards with a relative tolerance of ± 30% and (iii) retention time similar to the calibration standard ±0.1 min (SANTE, 2017)

2.3. Statistical analysis

Statistical analysis was performed using R software (version 3.4.0). Parabens and phenols levels in urine below detection limit were imputed following the maximum likelihood estimation method described in (EFSA, 2010). This method assumes that the data are distributed according to a certain parametric distribution and estimates the parameters of this distribution so that the

probability of obtaining the observed sample is maximized. A log-normal distribution was assumed for bisphenols and parabens levels in urine.

A descriptive analysis of all the variables was performed. Qualitative variables were described by absolute frequencies and percentages. Quantitative variables were summarised by their median and range. Additionally, levels in urine were summarized by calculating the minimum, 25th, 50th, 75th and 95th percentiles, arithmetic mean (AM), geometric mean (GM), maximum and standard deviation. Spearman correlation test was employed to explore correlations between bisphenols and parabens levels in urine.

Simple and multiple linear regression models were built to assess the relationship between the concentration of each phenol and paraben in urine with sociodemographic, dietary (including package food consumption) and use of cosmetic products variables. The logarithmic transformation of parabens and bisphenols levels in urine was considered to get the normality of the response variables. Multiple regression models were built considering those variables with a p-value lower than 0.05 in simple regression models and following a backward variable selection procedure based on the Bayesian Information Criterion (BIC) (Konishi et al., 2003).

2.4. Exposure and risk assessment

Biomonitoring Equivalents (BE) are one of the guidance values used for risk assessment studies (Hays et al. 2018; Aylward et al, 2011). Krishnan et al. (2010) has described BE value for BPA. In this case, the Hazard Quotient (HQ) was calculated as the ratio between biomarker concentration (95^{th} percentile) to specific BE value (Krishnan et al, 2010). A HQ > 1 indicated risk (EFSA, 2013).

For the rest of the studied compounds, in which the internal guidance values are not available, reverse dosimetry has been applied in order to estimate bisphenols and parabens external exposure. Following this approach, the estimated daily intake (EDI) has been calculated according the following equation:

EDI (mg/ kg bw day) = (C (mg/l)·Vurine(l/day))/(F·BW(kg)) (Equation (1))

where C is the obtained concentration of bisphenols or parabens; V urine is the total urinary volume excreted in 24 h; F is the compound urinary excretion factor and BW is the reference body weight. The considered C was the percentile 95^{th} for each compound. The volume of urine for adults was 1.50 L according to Dirtu et al. (2013). There are no data available for the urinary excretion factor of each compound (MP, EP and PP) in the bibliography; in this case 1 and 0.25 were considered as optimistic and pessimistic scenario, respectively. The body weight value considered was 70 kg (WHO, 2006).

The risk assessment was estimated depending on the reference values available for each compound. EFSA expert panel (2004) established a group ADI (Admissible Daily Intake) of 0-10 mg/kg bw·day for MP and EP but could not recommend an ADI for PP. Consequently, for PP we have calculated a safety margin as described by Boberg et al. (2010).

In the case of MP and EP, the risk assessment was estimated using the Hazard Quotients (HQ) as a risk descriptor, which were calculated as follows:

HQ = (∑EDI calculated)/ (ADI) (Equation (2))

where EDI is the estimated daily intake in this study and ADI is the admissible daily intake in which adverse effects are not expected. The values for each ADI were retrieved from EFSA reports (EFSA, 2004). EDI was compared to ADI calculating the HQ which is the ratio of the potential external exposure (EDI) to the levels at which no adverse effects are expected (ADI). Calculated daily intake was considered safe if HQ < 1 (EFSA, 2013; Katsikantami et al 2019).

On the other hand, the margin of safety has been used as a risk descriptor when ADI value was not available, which were calculated as follows:

MS = (NOAEL)/ (EDI calculated) (Equation (3))

where EDI is the estimated daily intake in this study and NOAEL is the no-observed adverse effect level

3. Results

3.1. Parabens and bisphenols urine levels

A total of 180 lactating mothers were selected to participate in the study. Urine specimens were obtained from 103 mothers .All mothers completed the various questionnaires. The studied personal and lifestyle variable results are presented in Table 1. Table SD-I describes food consumption by groups taking into account diet administered questionnaires. Frequency of use of cosmetic products in breastfeeding mothers are shown in Table SD-II.

The occurrence of parabens and bisphenols in breastfeeding mother urine are presented in Table 2. Two parabens (MP and EP) and one bisphenol (BPA) were present at high frequencies between 73-92%. 95th percentile was 617 µg/g (MP), 35.2 µg/g (EP) and 9.7 µg/g (BPA). Histograms of MP, EP, PP and BPA concentrations are depicted in Figure 1.

Taking into account that BP, BPF and BPS presented frequencies of detection lower than 40%, these substances have not been considered for regression studies.

Table SD-VII presents the correlation between paraben compounds. PP was significantly correlated to EP (p-value ≤0.001). Mothers with higher PP urinary levels also presented more EP in their urine. However, any correlation was found for bisphenols. As it can be observed in table SD-VIII, BPA was not correlated with any other phenol.

3.2. Factors of influence on parabens and BPA levels

The results of the simple regression model between the independent variables with the levels of parabens and bisphenols in urine are shown in tables SD-III, IV, V and VI. The parameters which showed the lowest p-values (≤ 0.05) using simple regression model were studied using a multiple regression model. As observed in tables SD-III, IV, V and VI, simple regression models showed some associations, which were later not confirmed in multiple regression models. This is the case of consuming package products and daily use of parfums for MP and BPA and applying skin care products for PP and BPA.

Table 3 shows the results for the variables for which we have found positive association using multiple regression model. Cosmetic products such as parfums showed high correlation for two compounds (MP and BPA). The mothers who used perfumes several times a week presented higher levels of MP than mothers who never used perfumes. Similarly, mothers who used perfumes daily or monthly also showed higher levels of BPA in their urine than mothers who never use these products (see Table 3).

3.3. Exposure and risk assessment for breastfeeding mothers

Exposure and risk assessment have been studied for one bisphenol (BPA) and three parabens (MP, EP and PP) due to their detection frequency was higher than 40% in the studied breastfeeding mothers.

Table 4 shows the estimated daily intakes (EDI) of different high detected parabens and BPA. EDIs for the sum of MP and EP was 0.0108 mg/kg·day (in an optimistic scenario) and 0.0434 mg/kg·day (in a pessimistic scenario). Both EDIs are lower than the ADI reported by EFSA (10 mg/kg·day for the sum of MP and EP). Consequently, HQs in both scenarios are two orders of magnitude lower than 1, which means that the exposure do not present risk for the mothers health. Table 4 lists safety margin for PP in the optimistic scenario. Considering a NOAEL value of 6.5 mg/kg·day (Boberg et al 2010), the margin of safety estimated in this study was 15476. Regarding to BPA, for which exists a BE of 2 mg L^{-1} (Krishnan et al. 2010), the calculated hazard coefficient was 0.0049. Consequently, no risk was derived from the estimated exposure.

4. Discussion

Table 5 presents urine´s paraben and bisphenol concentrations for different populations around the world. Higher BPA geometric mean concentrations have been described in American women (2.04 µg/g creatinine) and Australian pregnant women (1.95 µg/g creatinine) than in the present study (0.94 µg/g creatinine). Regarding to the exposition of bisphenols in breastfeeding women´s urine, to our knowledge, Hines et al. (2015) is the only study available in the literature anddetected higher concentrations in 75th percentile (4.1 ng mL^{-1}) than our breastfeeding mothers (2.9 ng mL^{-1}).

Previous worldwide studies, one in Australia (Heffernan et al., 2016) and other in Norway (Sakhi et al., 2018), studied the levels of BPS and BPF in urine of pregnant and no-pregnant women, respectively. BPS and BPF were not found on Australian pregnant women´s urine (Heffernan et al., 2016). However, Sakhi et al. (2018) reported geometric means of 0.11 ng mL^{-1} and 0.08 ng mL^{-1} for BPS and BPF, respectively, in Norwegian non-pregnant women, that are higher than in our study..

Different studies have reported the concentrations of certain parabens in the urine of adults (Quiros-Alcalá et al., 2018) and women (Sakhi et al., 2018; Jimenez-Díaz et al., 2016; Pollack et al., 2016) in USA, China, Norway and Tunis. However, information on the paraben concentrations in the urine of vulnerable populations, such as pregnant or lactating women, is limited. To our knowledge, Hines et al. (2015) published the only study for breastfeeding mothers, in which they investigated different parabens concentrations in lactating mothers 'urine from 2004 to 2005 in USA. All four parabens (MP, EP, PP and BP) detected in 34 lactating mothers were also found in our breastfeeding mother population. In all cases the levels found were higher than in our study (see Table 5).

In simple linear regression models, no association was seen between the variables age of participant, "having their first children pregnancy", BMI (body mass index) and urinary BPA concentration. Similar to our results, Philips et al. (2018) did not find association between maternal age and bisphenol concentrations in multivariable associations. Other studies have found association between maternal BMI, smoking, maternal age, lower education with higher BPA levels using different methodologies (Callan et al 2013; Arbuckle et al 2014; Valvi et al 2015). Jiménez-Díaz et al. (2016) found association, using Mann–Whitney U test, between age and urinary levels of PP, probably due to a more frequent use of personal care products in young adult women compared to older women. They also found that women working outside home

showed higher urinary levels of PP than homeworkers, suggesting a greater use of cosmetics and processed food in women working outside home.

Both the use of personal care products (PCPs) and consumption of food were the main predictors of bisphenols and paraben levels in the multiple linear regression model. Use of PCPs is one of the major sources of exposure to parabens. For PCPs, we found positive associations between use of parfums and concentrations of MP and BPA. However, we did not find associations with other PCPs such as skin care, sunscreen, deodorants or hair colour for the studied substances. In contrast, Sakhi et al. (2018) found positive associations between use of hand soap, face cream, hair products and concentrations of most environmental phenols and parabens in mothers and children in Norwegian population

When exploring dietary determinants, some food groups were significantly associated with urinary MP (fish), EP (legumes and cereals) and BPA (fruits) levels. However, other studies have not identified a clear pattern with any of the food groups consumed and the studied environmental phenols (Sakhi et al. 2018). Although some studies have shown that consumption of canned food is important predictor of urinary BPA, in the present work, we have not found this association (Sakhi et al. 2018; Philips et al 2018). Positive, though weak associations, were found between BPA and fruit consumption. The main route of BPA exposure is believed to be dietary and the major food sources are suggested to be canned products such as tinned meat, vegetables or fruits (Callan et al 2013). Unfortunately, in our questionnaire, we do not have information about the percentage of tinned fruit consumed by our population. We observed slightly higher concentrations of MP and EP in breastfeeding mothers who had eaten fishing products and legumes/cereals, respectively. Probably, this is due to parabens have mainly been used as antimicrobial preservatives in these food products (Sakhi et al 2018). Jiménez-Díaz et al. (2016), described that cereal consumption was positively associated with urinary levels of MP, probably because of the migration of this compound from antibacterial plastic packaging. However, Philips et al. (2018) found lower concentrations of BPS in pregnant woman who had eaten a large quantity of grains, while for women with a high consumption of fish and shellfish higher BPA concentrations were found (Philips et al. 2018).

In agreement with our results, Aker et al. (2018) have found correlation between parabens. They found strongly correlation between MP and PP, and moderately in the case of BP and EP. In contrast, for bisphenols any correlation has been found in the literature and in this study. Sakhi et al. (2018) did not find correlation between BPA and any environmental phenols.

Like in our study, Sakhi et al. (2018) estimated daily intakes of different environmental phenols and parabens in mothers and children. In agreement with our results, none of the participants exceeded the ADI for BPA, MP and EP as established by EFSA. Median daily intake for BPA was calculated in Australian pregnant women population by Callan et al. (2013). In that study, the estimated intake (range 0.01-0.14 μg/kg·day) obtained was 3 orders of magnitude below the European tolerable daily intake for BPA and US EPA reference dose of 50 μg/kg·day (Callan et al. 2013). Regarding to parabens, the sum of MP and EP obtained in a pessimistic scenario in this study was 0.434 mg/kg·day. This value is lower than the ADI established by EFSA (10 mg/kg·day) (EFSA, 2004). As described in table 4, calculated daily intakes are safe for breastfeeding mothers, since HQ is three orders of magnitude lower than 1 for all the studied substances.

5. Conclusions

Three bisphenols (BPA, BPF and BPS) and four parabens (MP, EP, PP and BP) were found in urine from breastfeeding Spanish mothers. BPA was the highly detected bisphenol, with concentrations ranging from values <LOQ to 40 μg/g creatinine. Low frequencies of detection have been observed for BPF and BPS. MP and EP were the most detected parabens in urinary samples, with concentrations in 95th percentile of 617 μg/g (MP) and 35.2 μg/g (EP)..

Use of parfums was associated with higher concentrations of MP and BPA. Two parabens (MP and EP) and one bisphenol (BPA) showed an association with some food groups consumption such as fishing products, legumes, cereals and fruits.

In general, the estimated exposure are three orders of magnitude lower than the reference values for risk assessment, consequently, it can be concluded that there is no risk for the studied mothers.

Acknowledgements

The authors would like to thank the work of Eva Villoldo, responsible for the collection of samples, who performed work with great efficiency. The study would not have been possible without the participating mothers and the effort and enthusiasm of the Division of Neonatology of the Pediatric Department at the University and Polytechnic Hospital La Fe. We want to particularly acknowledge the donors and the IBSPCV BioBank (PT13/0010/0064) integrated in the Spanish National Biobank Network and the Valencian Biobanking Network for their collaboration.

Funding sources

This study is part of the BETTERMILK project funded by the Generalitat Valenciana (GV/2015/008).

References

Adoamnei E., Mendiola J., García M., Soria F., Duràn LM., Fernandez M. Olea N. Jorgensen N., Swan S., Cantero A, 2018. Urinary concentrations of parabens and reproductive parameters in young men. Sci Total Environ. 621:201-209

Aker, L. Johns, T.F. McElrath, D.E. Cantonwine, B. Mukherjee, J.D. Meeker, 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study, Environment International. (113), 341-349.

Arbuckle, T.E., Davis K., Marro L., Fisher M., Legrand M., Leblanc A., Gaudreau E., Foster WG, Choeurng V, Fraser WD., MIREC study group. 2014. Phthalate and bisphenol A exposure among pregnant women in Canada--results from the MIREC study. Environ Int. 2014,68:55-65.

Aylward L., Hays S., 2011. Consideration of dosimetry in evaluation onf ToxCast^{TM} data. Journ. Applied Toxicology, 31)8) 741-751.

Boberg J., Taxvig C., Christiansen S., Hass V.2010. Possible endocrine disprupting effects of parabens and their metabolites. Reproductive Toxicology (30) 301-312

Buckley J., Herring A., Wolff M. ,Calafat A., Engel S. 2016. Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's Environmental Health Study Environment International, (91) 350-356.

Callan A., Hinwood A., Heffernan A., Eaglesham G., Mueller J., Odland J., 2013. Urinary bisphenol A concentrations in pregnant women.Int J Hyg Environ Health. 216(6):641-4.

Carita C., Lee Hinwood, Heffernan A., Eaglesham G., Øyvind J.Odland 2013.Urinary bisphenol A concentrations in pregnant women. International Journal of Hygiene and Environmental Health, 216 (6) 641-644

Chen, D., Kannan K., Tan H., Zheng Z., Feng Y., Wu Y., Wdelka M., 2016. Bisphenol analogues other than BPA: environmental occurrence, human exposure and toxicity- A review. Environ. Sci. Technol. 50, 5438-5453.

Darbre P., Harvey P.,2008. Paraben esters: Review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussionof potential human health risks. J. Appl. Toxicol 28: 561–578.

Dirtu, A., Geens T., Dirinck E., Malarvannan G., Neels H., Van Gaal, L., 2013. Phthalate metabolites in obese individuals undergoing weight loss: Urinary levels and estimation of the phthalates daily intake. Envir. Int. 59, 344–353.

Dualde P., Pardo O., Corpas-Burgos F., Kukugowski J., Gormaz M., Vento M.,Pastor A., Yusà V., 2019. Biomonitoring of bisphenols A,F,S in human milk and probabilistic risk assesment for breastfed infants. Science of Total Environ 668, 797-805.

EFSA, 2004. Scientific opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a Request from the Commission related to para hydroxybenzoates (E 214-219). EFSA J. (2004) 83, 1-26.

EFSA, 2010. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA J. 8 (3), 96. https://doi.org/10.2903/j.efsa.2010.1557

EFSA 2013, International Frameworks dealing with human risk assessment on combined exposure to multiple chemicals. EFSA J. 11 (7), 3313. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812121190260464 5.html. acc [015/05/2017].

EFSA, 2015. Scientific opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA J. 13 (1), 3978.

EU, 2011. EUROPEAN COMMISSION, 2011. European commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Off.J.Eur. Union L12(2011)1. EU, 2018. COMMISSION REGULATION 2018/213 of 12 February 2018 on the use of bisphenol A in varnishes and coatings intended to come into contact with food and amending Regulation (EU) No 10/2011 as regards the use of that substance in plastic food contact materials.

Guo, C. Wu, D. Lu, S. Jiang, W. Liang, X. Chang, H. Xu, G. Wang, Z. Zhou, 2017. Urinary paraben concentrations and their associations with anthropometric measures of children aged 3 years, Environmental Pollution.(222) 307-314.

Hays S. ,Poddalgodab D., Maceyb K. , Aylwardc L. , Nongb A.,2018 Biomonitoring Equivalents for interpretation of urinary iodine. Regulatory Toxicology and Pharmacology 94 , 40–46

HBM4EU, 2017. Deliverable Report D 4.2. WP 4 Prioritisation and input to the Annual Work Plan. Available online at https://www.hbm4eu.eu/deliverables. (accessed 15.07.2018).

Heffernan AL, Thompson K, Eaglesham G, Vijayasarathy S, Mueller JF, Sly PD, et al. Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine. Talanta 151(2016), 224-233.

Hines E., Mendola P., Von Ehrenstein Os, Ye X, Calafat Am. Fenton Se, 2015. Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women. Reprod Toxicol. 54:120-8.

Jardim C., Melo P., Domingues S., Costa Q., Determinatio of parabens in urine samples by microextraction using packaging sorbent and ultra-performance liquid chromatography coupled totandem mass spectrometry. Journal of Chromatography B 974 (2015), 35-41.Jiménez-Díaz, F. Artacho-Cordón, F. Vela-Soria, H. Belhassen, J.P. Arrebola, M.F. Fernández, R. Ghali, A. Hedhili, N. Olea, Urinary levels of bisphenol A, benzophenones and parabens in Tunisian women: A pilot study, Science of The Total Environment. 562 (2016), 81-88

Katsikantamia I., Colosio C, Alegakis A., Tzatzarakis M., Vakonaki E. Rizos A., Sarigiannisd D., Tsatsakisb A., Estimation of daily intake and risk assessment of organophosphorus pesticides based on biomonitoring data – The internal exposure approach. Food and Chemical Toxicology 123, 57–71

Krishnan K., Gagné M., Nong A., Aylward LL., Hays SM., 2010. Biomonitoring Equivalents for bisphenol A (BPA). Regul Toxicol Pharmacol. 58(1):18-24.

Larsen, K., 1972.Creatinine assay by a reaction-kinetic principle. Clin. Chim. Acta 41,209–217.

Lee et al., 2017 J. Lee, K. Choi, J. Park, H. Moon, G. Choi, J.J. Lee, E. Suh, H. Kim, S. Eun, G. Kim, G.J. Cho, S.K. Kim, S. Kim, S.Y. Kim, S. Kim, S. Eom, S. Choi, Y.D. Kim, S. Kim. Bisphenol a distribution in serum, urine, placenta, breast milk, and umbilical cord serum in a birth panel of mother-neonate pairs. Sci. Total Environ., 626,1494-1501

Liao, C., Liu, F., Alomirah, H., Loi, Vu Duc, Mohd, M.A., Moon, H., Nakata, H., Kannan, K., 2012. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environ. Sci. Technol. 46, 6860–6866.

Philips, E.M., Jaddoe, V.W.V., Asimakopoulos, A.G., Kannan, K., Steegers, E.A.P., Santos, S., Trasande, L., 2018. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004-5. Environ. Res. 161, 562–572.

Pollack A., Perkins N., Sjaarda L., Mumford S., Schisterman E., 2016. Variability and exposure classification of urinary phenol and paraben metabolite concentrations in reproductive-aged women. Environmental Research, 151, 513-520.

Quirós-Alcalá L., Buckleyc J., Boyle M., 2018. Parabens and measures of adiposity among adults and children from the U.S. general population: NHANES 2007–2014. International Journal of Hygiene and Environmental Health (221)652–660.

Rochester and Bolden, 2015. J.R. Rochester, A.L. Bolden Bisphenol S and F: a systematic review and comparison of the hormonal activity of bisphenol a substitutes. Environ. Health Perspect., 123, 643-650.

Sakhi, A. Sabaredzovic, E. Papadopoulou, E. Cequier, C. Thomsen, Levels, variability and determinants of environmental phenols in pairs of Norwegian mothers and children, Environment International. 114 (2018), 242-251.

Sanchis Y., Coscollà C., Yusà V., 2017. Analytical strategies for organic food packaging contaminants. Journal of Chromatogr A 1490:22-46.

Sanchis Y., Coscollà C., Yusà V., 2019. Analysis of four parabens and bisphenols A, F, S in urine, using dilute and shoot and liquid chromatography coupled to mass spectrometry. Talanta (202) 42-50.

SANTE/11813/2017. European Commission Guidance Document on Analytical QualityControl and Method Validation Procedures for Pesticide Residues Analysis in Foodand Feed.

Sclumpf M.,Kypke K., Wittassek M., Angerer J., Mascher H., Mascher D., Vökt C., Biirchler M., Lichtensteiger W., 2010. Exposure patterns of UV filters, fragances, parabens, phthalates, organochlor pesticides, PBDEs, and PCBs in human milk: Correlation of UV filters with use of cosmetics. 81, 1171-1183.

Konish S, Kitagawa G., 2003. Asymptotic theory for information criteria in model selection—functional approach. Journal of Statistical Planning and Inference (114) 45-61.

Valvia D., Monfort N.,Ventura R., Casasa,M, Casas L., Sunyera,J., Vrijheid M., 2015. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. International Journl of Hygiene and Environmental Health 218, 220–231

WHO, 2006. The World Health Report 2006 - working together for health. https://www.who.int/whr/2006/en/ (accessed 08.11.2017)

464 Yang, Y., Guan, J., Yin, J., Shao, B., Li, H., 2014. Urinary levels of bisphenol analogues in residents
465 living near a manufacturing plant in South China. Chemosphere 112, 481–486

466 Yusa, V., Perez, R.,Sanchez A., Pardo O., Roca M, 2018. Exposure and risk assessment to arsenic
467 species in Spanish children using biomonitoring.Science of the Total Environment 628-629; 302-
468 309.

469 Yusa, V., Perez, R., Suelves, T., Corpas-Burgos, F., Gormaz, M., Dualde, P., Coscolla, C., Quiles, J.,
470 Roca, M., Vento, M., 2017. Biomonitoring of mercury in hair of breastfeeding mothers living in
471 the Valencian Region (Spain). Levels and predictors of exposure. Chemosphere (187) 106–113

472 Zhou, X., Kramer, J.P., Calafat, A.M., Ye, X., 2014. Automated on-line column-switching high-
473 performance liquid chromatography isotope dilution tandem mass spectrometry method for the
474 quantification of bisphenol A, bisphenol F, bisphenol S, and 11 other phenols in urine. J.
475 Chromatogr. B 944, 152–156

476 **Table 1.** Characteristics of the study population

Characteristics	n (%) (N=103)
Mother	
Number of children	
1	61 (59.22 %)
2	33 (32.04 %)
3 or more	9 (8.74 %)
Age (years)	34 (20 - 45)[a]
Weight before pregnancy (kg)	60 (42 - 92)[a]
Height (cm)	163 (150 - 184)[a]
BMI before pregnancy (kg/m²)	21.9 (16.6 – 35.4)[a]
Diet during pregnancy	
Yes	13 (12.62 %)
No	88 (85.44 %)
Country of birth	
Spain	88 (85.44 %)
Foreign	13 (12.62 %)
Place of residence	
Urban	75 (72.81 %)
Rural	17 (16.51 %)
Education level	
Only primary school	11 (10.68 %)
Secundary school	21 (20.39 %)
University	71 (68.93 %)
Occupational status	
Employed	88 (85.44 %)

Unemployed	15 (14.56 %)
Time worked outside the home (years)	9 (0 - 28)[a]
Use of cosmetics at work	
Yes	8 (7.77 %)
No	95 (92.23 %)
Breastfed	
Yes	70 (67.96 %)
No	29 (28.15 %)
Physical exercise	
3 or more days/week	15 (14.56 %)
1 or 2 days/week	17 (16.50 %)
Occasionally	44 (42.72 %)
Never	24 (23.30 %)
Smoker	
Yes	7 (6.8 %)
Ex-smoker	41 (39.81 %)
Never	55 (53.4 %)
Child	
Gestational age (weeks)	40 (35 - 41)[a]
Sex	
Boy	38 (36.89 %)
Girl	62 (60.19 %)
Weight (g)	3350 (2160 - 4350)[a]
Height (cm)	51 (46 - 55)[a]
Cranial perimeter (cm)	34 (32.5 - 37)[a]

Table 3. Results of the multiple linear regression model for log [parabens and phenols] levels in urine of breastfeeding mothers

Compounds	Variable	Estimated coefficients (95% CI)	Standard error	P-value
MP	Intercept	-25.5186 (-49.1037 - -1.9335)	11.6401	0.0347*
	Fishing products (g/month) (N=97)	0.0003 (0.00004 - 0.0006)	0.0004	0.0256*
	Parfums: several times a week (N=20)	2.6393 (0.7646 - 4.5139)	0.9252	0.0071*
EP	Intercept	4.7844 (2.1253 - 7.4434)	1.3386	0.0006*
	Legumes and cereals (g/month) (N=103)	0.0002 (0 – 0.0003)	0.0001	0.0239*
BPA	Intercept	-10.9894 (-19.9693 - -2.0095)	4.5179	0.017*
	Fruits (g/month) (N=97)	$3.29 \cdot 10^{-5}$ ($3.79 \cdot 10^{-6}$ – 0.0001)	$1.46 \cdot 10^{-5}$	0.0272*
	Parfums: daily (N=28)	1.0128 (0.2144 - 1.8111)	0.4017	0.0135*
	Parfums: sometimes in month (N=4)	1.7798 (0.1042 - 3.4554)	0.843	0.0376*

Table 4. Estimated daily intakes, hazard quotients, reference values and margin of safety.

MP and EP

P95[a] [mg/L]	$EDI_{optimistic\ (MP+EP)}$ [mg/kg·day]	$EDI_{pessimistic}$ (MP+EP) [mg/kg·day]	ADI [mg/kg·day]	$HQ_{optimistic}$	$HQ_{pessimistic}$
0.486 (**MP**)	0.0108	0.0434	10	0.001	0.004
0.0205 (**EP**)					

PP

P95[a] [mg/L]	$EDI_{optimistic}$ [mg/kg·day]	NOAEL[b] [mg/kg·day]	Margin of Safety
0.02	0.00042	6.5	15476

BPA

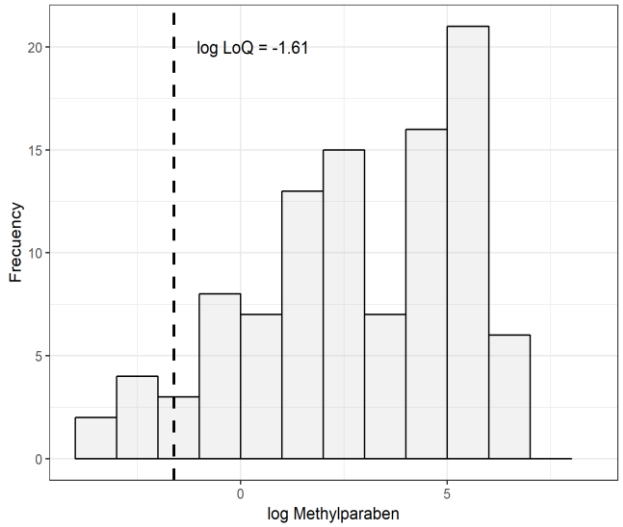
P95[a] [mg/L]	BE[c] [mg/L]	HQ
0.0097	2	0.0049

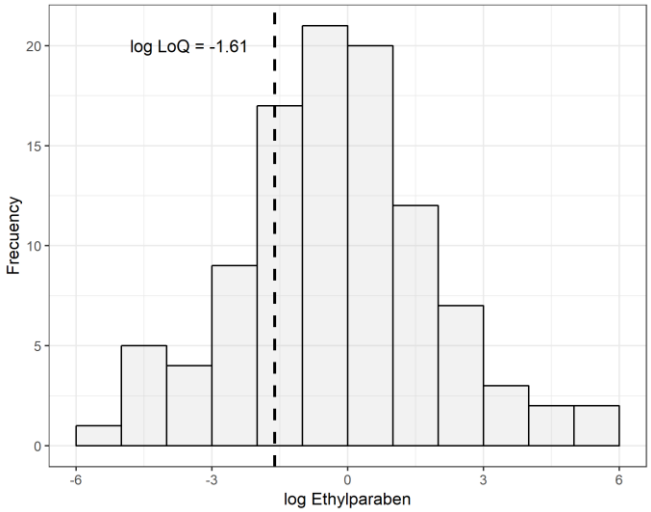
a: 95th Percentile, b: no-observed adverse effect, c: biomonitoring equivalent value.

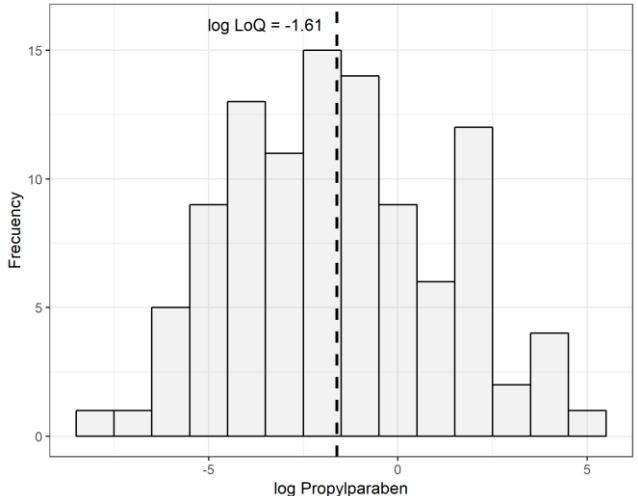
Table 5. Comparative data on levels of parabens and bisphenols A, F and S in urine in other studies

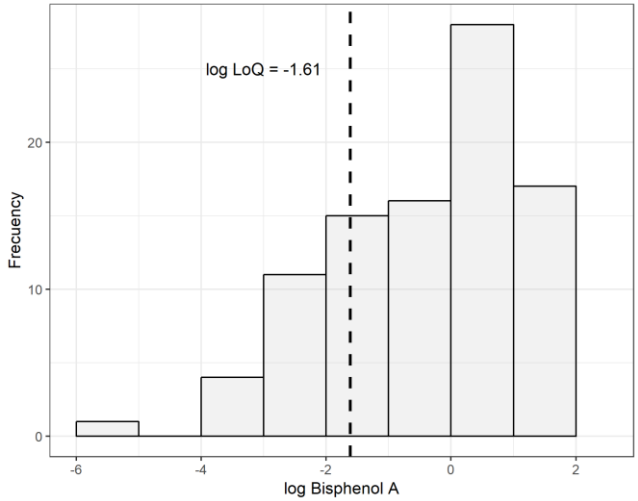
Compounds	Study	Country/location	Year	Population	N	DF (%)[a]	Age (years)	P75 (75th Percentile)	GM (geometric means)
MP									
	This study	Spain (Valencia)	2015	breastfeeding mothers	119	92	20-45	165 ng mL^{-1} (154.8 µg/g creat)	17.7 ng mL^{-1} (18.03 µg/g creat)
	Quirós-Alcalá et al., 2018	United States	2007-2014	adults	4730	>90	≥20	245.9 ng mL^{-1} (253.8 µg/g creat)	5.8 ng mL^{-1} (5.9 µg/g creat)
	Adoamnei E et al., 2018	Spain (Murcia)	2010-2011	Young men	215	60-92	18-23	40.8 ng mL^{-1} (27.3 µg/g creat)	11.2 ng mL^{-1} (8.8 µg/g creat)
	Sakhi A.K. et al., 2018	Norwegian	2012	women	48	-	32-56	171 (ng mL^{-1})	61.5 ng mL^{-1}
	Jiménez-Díaz I et al., 2016	Tunis	2012	Women	34	94	[<45, >45]	-	34.94 ng mL^{1}
	Pollack A et al.,2016	United States	2005-2007	women	509	-	[> 18,44]	146.9 ng mL^{1} (101.7 µg/g creat)	49.5 ng mL^{1} (37.2 µg/g creat)
	Hines E. et al., 2015	United States	2004-2005	breastfeeding mothers	34	100	-	266 ng mL^{1}	-
EP									
	This study	Spain (Valencia)	2015	breastfeeding mothers	119	73	20-45	2.6 ng mL^{-1} (2.63 µg/g creat)	0.78 ng mL^{-1} (0.79 µg/g creat)
	Quirós-Alcalá et al., 2018	United States	2007-2014	adults	4730	<50	≥20	6.7 ng mL^{-1} (6.6 µg/g creat)	-
	Adoamnei E et al., 2018	Spain (Murcia)	2010-2011	Young men	215	60-92	18-23	3.9 ng mL^{-1} (3 µg/g creat)	1.1 ng mL^{-1} (0.85µg/g creat)
	Sakhi A.K. et al., 2018	Norwegian	2012	women	48	-	32-56	18.6 ng mL^{-1}	3.34 ng mL^{-1}
	Jiménez-Díaz I et al., 2016	Tunis	2012	Women	34	68	[<45, >45]	-	1.77 ng mL^{-1}
	Pollack A et al.,2016	United States	2005-2007	women	509	-	[> 18,44]	4.8 ng mL^{1} 4.4(µg/g creat)	0.8 ng mL^{1} (0.6 µg/g creat)
	Hines E. et al., 2015	United States	2004-2005	breastfeeding mothers	34	<50	-	19.4 ng mL^{1}	-
PP	This study	Spain (Valencia)	2015	breastfeeding mothers	119	49	20-45	1.5 ng mL^{-1} (1.63 µg/g creat)	0.21 ng mL^{-1} (0.21 µg/g creat)
	Quirós-Alcalá et al., 2018	United States	2007-2014	adults	4730	>90	≥20	50.9 ng mL^{-1} (54.5 µg/g creat)	2.4 ng mL^{-1} (2.4 µg/g creat)
	Adoamnei E et al., 2018	Spain (Murcia)	2010-2011	Young men	215	60-92	18-23	3.7 ng mL^{-1} (2.6 µg/g creat)	0.64 ng mL^{-1} (0.5 µg/g creat)
	Sakhi A.K. et al., 2018	Norwegian	2012	women	48	-	32-56	25.7 ng mL^{-1}	5.38 ng mL^{-1}
	Jiménez-Díaz I et al., 2016	Tunis	2012	Women	34	71	[<45, >45]	-	3.06 ng mL^{-1}
	Pollack A et al.,2016	United States	2005-2007	women	509	-	[> 18,44]	48.6 ng mL^{1} (30.2 µg/g creat)	12.5 ng mL^{1} (9.4 µg/g creat)
	Hines E. et al., 2015	United States	2004-2005	breastfeeding mothers	34	>50	-	69 ng mL^{1}	-
BP									
	This study	Spain (Valencia)	2015	breastfeeding mothers	119	12	20-45	0.08 ng mL^{-1} (0.08 µg/g creat)	0.021 ng mL^{-1} (0.021 µg/g creat)
	Quirós-Alcalá et al., 2018	United States	2007-2014	adults	4730	<50	≥20	0.6 ng mL^{-1} (0.7 µg/g creat)	-
	Adoamnei E et al., 2018	Spain (Murcia)	2010-2011	Young men	215	9	18-23	-	-
	Sakhi A.K. et al., 2018	Norwegian	2012	women	48	-	32-56	0.48 ng mL^{-1}	0.15 ng mL^{-1}
	Jiménez-Díaz I et al., 2016	Tunis	2012	women	34	38	[<45, >45]	-	< 0.2 ng mL^{-1}
	Pollack A et al.,2016	United States	2005-2007	women	509	-	[> 18,44]	2.3 ng mL^{1} (1.7 µg/g creat)	0.3 ng mL^{1} (0.2 µg/g creat)
	Hines E. et al., 2015	United States	2004-2005	breastfeeding mothers	34	<50	-	4.6 ng mL^{1}	-
BPA									
	This study	Spain (Valencia)	2015	breastfeeding mothers	119	76	20-45	2.9 ng mL^{-1} (3.56 µg/g creat)	0.927ng mL^{-1} (0.94 µg/g creat)
	Pollack A et al.,2016	USA (Massachusetts)	2005-2007	women	509	-	[> 18,44]	6.8 ng mL^{-1} (4.8 µg/g creat)	1.7 ng mL^{-1} (2.04 µg/g creat)
	Hines E. et al., 2015	United States	2004-2005	breastfeeding mothers	34	80-90	-	4.1 ng mL^{-1}	-
	Carita A et al.,2013	Western Australia	2008-2011	Pregnant women	173	85	[25-39]	-	1.6 ng mL^{-1} (1.95 µg/g creat)
	Arbuckle T et al.,2014	Canada	2008-2011	Pregnant women	1788	88	[25-39]	-	1.02 ng mL^{-1}
	Heffernan A et al.,2016	Australia (Brisbane)	-	Pregnant women	30	100	>18	-	5 ng mL^{-1}
	Sakhi A.K. et al., 2018	Norwegian	2012	women	48	100	32-56	4.14 ng mL^{-1}	3.02 ng mL^{-1}
	Jiménez-Díaz I et al., 2016	Tunis	2012	women	34	65	[<45, >45]	1.40 ng mL^{-1}	0.44 ng mL^{-1}
	Buckley J. et al., 2016	United States	1998-2002	Pregnant women	479	86	-	2.30 ng mL^{-1}	1.25 ng mL^{-1}
BPS									
	This study	Spain (Valencia)	2015	breastfeeding mothers	119	20	20-45	0.17 µg L^{-1} (0.17 µg/g creat)	0.061 ng mL^{1} (0.062µg/g creat)
	Heffernan A et al.,2016	Australia (Brisbane)	-	Pregnant women	30	10	>18	-	-
	Sakhi A.K. et al., 2018	Norwegian	2012	women	48	42-48	32-56	0.26 ng mL^{-1}	0.11 ng mL^{-1}
BPF									
	This study	Spain (Valencia)	2015	breastfeeding mothers	119	20	20-45	0.135 ng mL^{1} (0.14 µg/g creat)	0.042 µg L^{-1} (0.043µg/g creat)
	Heffernan A et al.,2016	Australia (Brisbane)	-	Pregnant women	30	N.D	>18	-	-
	Sakhi A.K. et al., 2018	Norwegian	2012	women	48	4-15	32-56	< LOD	0.08 ng mL^{-1}

a: frequency of detection (%)


log LoQ = -1.61
Frecuency
log Methylparaben


log LoQ = -1.61
Frecuency
log Ethylparaben


log LoQ = -1.61
Frecuency
log Propylparaben


log LoQ = -1.61
Frecuency
log Bisphenol A

5. Conclusiones

Las principales conclusiones generales derivadas de la realización de los estudios descritos en esta Tesis Doctoral son las siguientes:

- Existe una necesidad de mayor regulación a nivel europeo en relación a las sustancias que migran desde los materiales no plásticos a los alimentos.
- Se observa una clara tendencia a la integración de los procedimientos de extracción y purificación en la preparación de la muestra, minimizando el uso de disolventes orgánicos y sustituyendo las técnicas de extracción convencionales por otras más novedosas, como PLE, SPME, FUSLE y QuEChERS.
- Las metodologías analíticas basadas en HPLC-MS/MS ofrecen una alta sensibilidad, permitiendo alcanzar límites de detección y cuantificación muy bajos. Esta característica es imprescindible para el control oficial de sustancias reguladas, tanto en los materiales como en los alimentos. Al mismo tiempo, es una herramienta muy útil para los estudios de *"biomonitoring*" de sustancias definidas *("análisis target").*
- El gran potencial que presenta el uso de la alta resolución (HRMS), gracias a su selectividad y su elevado poder de resolución (50000 FWHM), posibilita el desarrollo de métodos *target, postarget* o "*suspect screening*" para los contaminantes derivados de los MCA. Una tendencia clara, que se reforzará en los próximos años, es el uso de LC-HRMS para un análisis mucho más amplio de los contaminantes del envasado de alimentos.
- El estudio de evaluación del riesgo debido a la exposición a parabenos y bisfenoles realizados en orinas de madres lactantes nos han indicado que, en general, los niveles encontrados en la población estudiada en la Comunitat Valenciana no suponen un riesgo para la salud de las personas.

Las conclusiones específicas ya han sido recopiladas en cada uno de los capítulos. A continuación, se detallan agrupadas por los diferentes estudios:

Capítulo 1

- La estrategia analítica desarrollada combina el análisis cuantitativo de 8 PAAs de en utensilios de poliamida (*target*) con un cribado retrospectivo de distintas

familias de compuestos procedentes de los materiales en contacto con los alimentos (envasado), mediante el uso de UHPLC-HRMS.

- El método cuantitativo presenta límites de cuantificación menores de 2 μg kg^{-1} para la mayoría de los analitos, con recuperaciones entre el 60 y el 120 % y precisiones menores del 15%, lo que lo hace útil para el control oficial.
- El potencial analítico de la alta resolución, la masa exacta y la adquisición en barrido completo con y sin fragmentación, permite la comparación con bases de datos para la identificación inicial de posibles contaminantes no incluidos en el método (análisis retrospectivo).
- La aplicación del método desarrollado en muestras reales combinando el análisis *target* y el *postarget* hacen de esta estrategia una prometedora herramienta para estudios de exposición a contaminantes procedentes del envasado y de los materiales.

Capítulo 2

- Se ha desarrollado una estrategia analítica basada en UHPLC-HRMS que permite el análisis comprensivo (*target* y *postarget*) de fotoiniciadores y aminas en distintos envases para alimentos y que puede utilizarse en la monitorización de estas sustancias.
- El método cuantitativo se ha desarrollado para 10 fotoiniciadores y 8 aminas, presentando un límite de cuantificación entre 0.5-5 μgkg^{-1} para los fotoiniciadores y 2-2.5 μgkg^{-1} para las aminas, con recuperaciones entre el 72 y el 120%, con precisiones menores del 20 % en dos tipos de simulantes (B y D1).
- Además de la masa exacta y del perfil isotópico, la identificación de las sustancias *target* y *postarget* (análisis retrospectivo) mejora utilizando los fragmentos generados en la celda de colisión (HCD) del Orbitrap. Sin embargo, es necesaria una optimización previa de la energía de colisión.
- El análisis *postarget* realizado sobre muestras reales mediante comparación con una base de datos de 87 sustancias, posibilitó la identificación tentativa de nuevos compuestos presentes en los materiales como perfluorados y retardantes de llama no incluidos inicialmente en el análisis dirigido.

Capítulo 3

- Se ha desarrollado una metodología rápida y sensible para la determinación de bisfenoles (BPA, BPF y BPS) y parabenos (MP, EP, PP y BP) en orina.
- El uso de una fuente APCI en el análisis LC-MS / MS reduce el efecto de la matriz y aumenta la sensibilidad del método para los bisfenoles, especialmente para el bisfenol A, obteniendo límites de detección mucho más bajos con fuente APCI.
- La preparación de la muestra es rápida y no requiere disolventes adicionales. La combinación de la preparación genérica de la muestra y la sensibilidad del análisis LC-MS/MS muestra su utilidad para futuros estudios de biomonitorización en orina humana.

Capítulo 4

- Tres bisfenoles (BPA, BPF y BPS) y cuatro parabenos (MP, EP, PP y BP) fueron analizados en la orina de madres lactantes en la Comunitat Valenciana. El BPA y el MP fueron las sustancias con mayores frecuencias de detección, 76% y 92% respectivamente. Compuestos análogos al BPA, cómo son el BPF y BPS, presentan baja frecuencia de detección FD =20%. Los parabenos EP y PP se detectaron con una frecuencia de detección del 73 y 49 % respectivamente, el BP presentó una FD del 12%.
- En la población de mujeres estudiada, el BPA con concentración media de 0.94 µg/g de creatinina presenta niveles más bajos que los descritos en la literatura para poblaciones similares. El MP, EP y PP con concentraciones medias de 18 µg/g, 0.8 µg/g y 0.2 µg/g respectivamente, tienen niveles inferiores a otro estudio realizado en EE. UU. para el mismo tipo de población.
- En este estudio, se ha observado una asociación entre el uso de productos de cuidado personal y el consumo de alimentos con una mayor concentración a bisfenoles y parabenos. El uso de perfumes se asoció con mayores concentraciones de MP y BPA. Dos parabenos (MP y EP) y un bisfenol (BPA) mostraron una asociación con el consumo de algunos grupos de alimentos, como productos de pesca, legumbres, cereales y frutas.

- La exposición estimada ha resultado ser de tres órdenes de magnitud inferior a los valores de referencia basados en salud, por tanto, se puede concluir que no hay riesgo para la población de madres estudiada.

Bibliografía

BIBLIOGRAFÍA

ADOAMNEI E., MENDIOLA J., GARCÍA M., SORIA F., DURÀN LM., FERNANDEZ M. OLEA N. JORGENSEN N., SWAN S., CANTERO A, 2018. Urinary concentrations of parabens and reproductive parameters in young men. The Science of the total Environment. 621, 201-209

AKER L., JOHNS T., MCELRATH D., CANTONWINE B., MUKHERJEE J., MEEKER D., 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study, Environment International 113, 341-349.

ALABI, A., CABALLERO-CASERO, N. and RUBIO, S., 2014. Quick and simple sample treatment for multiresidue analysis of bisphenols, bisphenol diglycidyl ethers and their derivatives in canned food prior to liquid chromatography and fluorescence detection. Journal of Chromatography A.1336, 23-33

ALNAIMI M., SABAH, ELOUADI, B. and KAMAL, I., 2007. A Study on Some Characteristics of Low Density Polyethylene Produced by QAPCO. DOI: 10.13140/2.1.1206.8640.

ANASTASSIADES, M., LEHOTAY, S., ÅTAJNBAHER, D. and SCHENCK, F., 2003. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and Dispersive Solid-Phase Extraction for the Determination of Pesticide Residues in Produce. Journal of AOAC International 86 (2), 412-31

ANGERER, J., EWERS, U. and WILHELM, M., 2007. Human biomonitoring: State of the art. International Journal of Hygiene and Environmental Health 210 (3-4), 201-28

ANZANO, J., LASHERAS, R., BONILLA, B. and CASAS, J., 2008. Classification of polymers by determining of C1:C2:CN:H:N:O ratios by laser-induced plasma spectroscopy (LIPS). Polymer Testing 27(6), 705-710

ARBUCKLE, T.E., WEISS, L., FISHER, M., HAUSER, R., DUMAS, P., BÉRUBÉ, R., NEISA, A., LEBLANC, A., LANG, C., AYOTTE, P., WALKER, M., FEELEY, M., KONIECKI, D. and TAWAGI, G., 2015. Maternal and infant exposure to environmental phenols as measured in multiple biological matrices. Science of the Total Environment 508, 575-84

ARBUCKLE, T.E., DAVIS K., MARRO L., FISHER M., LEGRAND M., LEBLANC A., GAUDREAU E., FOSTER WG, CHOEURNG V, FRASER WD., MIREC study group, 2014. Phthalate and bisphenol A exposure among pregnant women in Canada--results from the MIREC study. Environment International, 68, 55-65.

ARVANITOYANNIS, I. and BOSNEA, L, 2004, Migration of Substances from Food Packaging Materials to Foods, Critical Review of Food Science and Nutrition 44(2),63-76.

AYLWARD L., HAYS S.,2011. Consideration of dosimetry in evaluation of ToxCast™ data, Journal of Applied Toxicology 31 (8),741-751.

AZNAR, M., RODRIGUEZ-LAFUENTE, A., ALFARO, P. and NERIN, C., 2012. UPLC-Q-TOF-MS analysis of non-volatile migrants from new active packaging materials. Analytical and Bioanalytical Chemistry 404(6), 1945-1957.

AZNAR, M., DOMEÑO, C., NERÍN, C. and BOSETTI, O., 2015. Set-off of non volatile compounds from printing inks in food packaging materials and the role of lacquers to avoid migration. Dyes and Pigments 114, 85-92

BLEDZKA, D., GROMADZISKA, J. and WASOWICZ, W., 2014. Parabens from environmental studies to human health. Environment International 67, 27-42

BACH, C., DAUCHY, X., CHAGNON, M. and ETIENNE, S., 2012. Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: A source of controversy reviewed. Water Research 46(3),571-83

BALLESTEROS, V., COSTA, O., IÑIGUEZ, C., FLETCHER, T., BALLESTER, F. and LOPEZ-ESPINOSA, M., 2017. Exposure to perfluoroalkyl substances and thyroid function in pregnant women and children: A systematic review of epidemiologic studies, Environment International 99,15-28 .

BALLESTEROS-GÓMEZ, A., RUBIO, S. and PÉREZ-BENDITO, D., 2009. Analytical methods for the determination of bisphenol A in food. Journal of Chromatography A, 1216 (3), 449-469.

BALLESTEROS-GÓMEZ, A., RUBIO, S. and VAN LEEUWEN, S., 2010. Tetrahydrofuran-water extraction, in-line clean-up and selective liquid chromatography/tandem mass spectrometry for the quantitation of perfluorinated compounds in food at the low picogram per gram level. Journal of Chromatography A, 1217(38) 5913-5921.

BARR, D., Y WANG, Richard and L NEEDHAM,Larry, 2005. Biologic Monitoring of Exposure to Environmental Chemicals Throughout the Life Stages: Requirements and Issues for Consideration for the National Children's Study. Environment and Health Perspective 113(8), 1083–1091

BESER, M.I., PARDO, O., BELTRÁN, J. and YUSÀ, V., 2019. Determination of 21 perfluoroalkyl substances and organophosphorus compounds in breast milk by liquid chromatography coupled to orbitrap high-resolution mass spectrometry. Analalytical Chimica Acta 1049,123-132.

BIGNARDI, C., CAVAZZA, A., CORRADINI, C. and SALVADEO, P., 2014. Targeted and untargeted data-dependent experiments for characterization of polycarbonate food-contact plastics by ultra high performance chromatography coupled to quadrupole orbitrap tandem mass spectrometry. Journal of Chromatography A. 1372C, 133-144.

BISHOP, C.A., 2011. 11 - Polymer Coating Basic Information. Oxford: William Andrew Publishing.

BOBERG, J., TAXVIG, C., CHRISTIANSEN, S. and HASS, U., 2010. Possible endocrine disrupting effects of parabens and their metabolites. Reproductive Toxicology 30(2),301-12

BREDE, C., SKJEVRAK, I. and HERIKSTAD, H., 2003. Determination of primary aromatic amines in water food simulant using solid-phase analytical derivatization followed by gas chromatography coupled with mass spectrometry Journal of Chromatography A 983(1-2),35-42

BUCK, R., FRANKLIN, J., BERGER, U., M CONDER,J ason, COUSINS, I., DE VOOGT, P., JENSEN, A., KANNAN, K., A MABURY,Scott and VAN LEEUWEN, S., 2011. Perfluoroalkyl and Polyfluoroalkyl Substances in the Environment: Terminology, Classification, and Origins. Integration and Environment Assessment Management 7(4), 513-41.

BUCKLEY J., HERRING A., WOLFF M. , CALAFAT A., ENGEL S. 2016. Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's Environmental Health Study. Environment International, (91) 350-356.

CACHO, J.I., CAMPILLO, N., VIÑAS, P. and HERNÁNDEZ-CÓRDOBA, M., 2012 Determination of alkylphenols and phthalate esters in vegetables and migration studies from their packages by means of stir bar sorptive extraction coupled to gas chromatography–mass spectrometry. Journal of Chromatography A 1241, 21-7.

CALLAN A., HINWOOD A., HEFFERNAN A., EAGLESHAM G., MUELLER J., ODLAND J., 2013. Urinary bisphenol A concentrations in pregnant women. International Journal of Hygiene and Environmental Health 216(6), 641-4.

CAMPANELLA G., GHAANI M., QUETTI G., FARRIS S., 2015. On the origin of primary aromatic amines in food packaging materials. Trends in Food Science & Technology 46, 137-143.

CAMPOS CAMPOS 2017, Migración de los distintos componentes de los envases, Toxicología de aditivos y materiales de empaque 4, 15-28.

CANELLAS, E., NERÍN, C., MOORE, R. and SILCOCK, P., 2010. New UPLC coupled to mass spectrometry approaches for screening of non-volatile compounds as potential migrants from adhesives used in food packaging materials. Analytical Chimica Acta. 666(1-2),62-9.

CAO, X., 2010. Phthalate Esters in Foods: Sources, Occurrence, and Analytical Methods. Comprehensive Reviews in Food Science and Food Safety 9(1), 21-43

CARITA C., HINWOOD L., HEFFERNAN A., EAGLESHAM G., ØYVIND J. ODLAND, 2013. Urinary bisphenol A concentrations in pregnant women. International Journal of Hygiene and Environmental Health, 216 (6) 641-644

CASTILLO, R., BIEDERMANN, M., RIQUET, A. and GROB, K., 2013. Comprehensive on-line HPLC-GC for screening potential migrants from polypropylene into food: The effect of pulsed light decontamination as an example. Polymer Degradation and Stability 98, 1679-1687

CINELLI, G., AVINO, P., NOTARDONATO, I., CENTOLA, A. and RUSSO, M.V., 2014. Study of XAD-2 adsorbent for the enrichment of trace levels of phthalate esters in hydroalcoholic food beverages and analysis by gas chromatography coupled with flame ionization and ion-trap mass spectrometry detectors. Food Chemistry 146,181-7.

CINELLI, G., AVINO, P., NOTARDONATO, I., CENTOLA, A. and RUSSO, M.V., 2013. Rapid analysis of six phthalate esters in wine by ultrasound-vortex-assisted dispersive liquid–liquid micro-extraction coupled with gas chromatography-flame ionization detector or gas chromatography–ion trap mass spectrometry. Analytical Chimica Acta 769,72-8.

CD 2011/8/EU. COMMISSION DIRECTIVE 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards the restriction of use of Bisphenol A in plastic infant feeding bottles. Official Journal of the European Union L 26/11.

CRISTINA JARDIM, V., DE PAULA MELO, L., SOARES DOMINGUES, D. and COSTA QUEIROZ, M.E., 2015. Determination of parabens in urine samples by microextraction using packed sorbent and ultra-performance liquid chromatography coupled to tandem mass spectrometry. Journal of Chromatography B Analytical Technololgies in the Biomedical and Life Science 974, 35-41.

DALLAIRE, R., DEWAILLY, E., PEREG, D., DERY, S. and AYOTTE, P., 2009. Thyroid Function and Plasma Concentrations of Polyhalogenated Compounds in Inuit Adults. Environment and Health Perspectives 117(9),1380-6.

DARBRE P., P.W.H., 2008. Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks, Journal of Applied Toxicology 28, 561-578

DAWIDOWICZ A., TYPEK R, DYBOWSKI M., NOWAKOWSKI P.,2019. Does the increase of radiation energy really reduce the risk of photoinitiator migration from polygraphic varnish to packed product? The influence of UV radiation doseonthemigrationof4-phenylbenzophenone frompolyacrylate varnish in food packaging. Food packaging and Shelf life 20, 100308.

DE ANDRADE, F.I., FLORINDO GUEDES, M.I., PINTO VIEIRA, ÍG., PEREIRA MENDES, F.N., SALMITO RODRIGUES, P.A., COSTA MAIA, C.S., MARQUES ÁVILA, M.M. and DE MATOS RIBEIRO, L., 2014. Determination of synthetic food dyes in commercial soft drinks by TLC and ion-pair HPLC. Food Chemistry 157,193-8.

DELMA L, MICHILG N, GAGGIOTTI M, GUADALUPEC, HORACIO R, REPETTI M, 2018. Determination of glyphosate, AMPA and glufosinate in dairy farm water from Argentina using a simplified UHPLC-MS/MS method. Science of the Total Environment 645,34–43

DE TONI, L., TISATO, F., SERAGLIA, R., ROVERSO, M., GANDIN, V., MARZANO, C., PADRINI, R. and FORESTA, C., 2017. Phthalates and heavy metals as endocrine disruptors in food: A study on pre-packed coffee products. Toxicology Reports 4 ,234-239.

DEMAID, A., SPEDDING, V. and ZUCKER, J., 1996. Classification of plastics materials. Artificial htelligence in Engineering 10, 9-20

D'EON, J.C., HURLEY, M.D., WALLINGTON, T.J. and MABURY, S.A., 2006. Atmospheric Chemistry of N-methyl Perfluorobutane Sulfonamido ethanol, C4F9SO2N(CH3) CH2CH2OH Kinetics and Mechanism of Reaction with OH. Environmental science & technology, 40(6), 1862-1868.

DEREUMEAUX, C., SAOUDI, A., PECHEUX, M., BERAT, B., DE CROUY-CHANEL, P., ZAROS, C., BRUNEL, S., DELAMAIRE, C., LE TERTRE, A., LEFRANC, A., VANDENTORREN, S. and GULDNER, L., 2016. Biomarkers of exposure to environmental contaminants in French pregnant women from the Elfe cohort in 2011. Environment International 97,56-67.

DIRTU, A., GEENS T., DIRINCK E., MALARVANNAN G., NEELS H., Van GAAL, L., et al., 2013. Phthalate metabolites in obese individuals undergoing weight loss: Urinary levels and estimation of the phthalates daily intake. Environment International 59, 344–353.

DODSON, R., NISHIOKA, M., J STANDLEY, Laurel, PEROVICH, L., BRODY, J. and RUDEL, R., 2012. Endocrine Disruptors and Asthma-Associated Chemicals in Consumer Products. Environment and Health Perspectives 120(7),935–943.

DUALDE P., PARDO O., CORPAS-BURGOS F., KUKUGOWSKI J., GORMAZ M., VENTO M., PASTORA., YUSÀ V.,2019. Biomonitoring of bisphenols A, F, S in human milk and probabilistic risk assesment for breastfed infants. Science of the Total Environment 668, 797-805.

DUGO, G.M., FOTIA, V., LO TURCO, V., MAISANO, R., POTORTÌ, A.G., SALVO, A. and DI BELLA, G., 2011. Phthalate, adipate and sebacate residues by HRGC-MS in olive oils from Sicily and Molise (Italy). Food Control 22(6), 982-988

DUTY, S.M., SINGH, N.P., SILVA, M.J., BARR, D.B., BROCK, J.W., RYAN, L., HERRICK, R.F., CHRISTIANI, D.C. and HAUSER, R., 2003. The Relationship between Environmental Exposures to Phthalates and DNA Damage in Human Sperm Using the Neutral Comet Assay. Environmental health perspectives**,** 111(9) 1164-1169.

ECA 2017. EUROPEAN CHEMICALS AGENCY. Inclusion of substances of very high concern in the Candidate list for eventual inclusion in Annex XIV. Helsinki, 04. 01. 2017 .

EFSA 2004a, EUROPEAN FOOD SAFETY AUTHORITY. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a Request from the Commission related to para hydroxybenzoates (E 214-219). The EFSA Journal 83, 1-26.

EFSA 2004b, EUROPEAN FOOD SAFETY AUTHORITY. Statement of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel) on the reclassification of some phthalates for consistency with the new SCF guidelines for food contact materials. The EFSA Journal 110, 1-27.

EFSA 2005a, EUROPEAN FOOD SAFETY AUTHORITY. Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to di-butylphthalate (DBP) for use in food contact materials, Question N°EFSA-Q-2003-192, EFSA Journal 242,1-17

EFSA 2005b, EUROPEAN FOOD SAFETY AUTHORITY. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to butylbenzylphthalate (BBP) for use in food contact materials, Question N°EFSA-Q-2003-190, EFSA Journal 241(2005b)1-14.

EFSA 2005c, EUROPEAN FOOD SAFETY AUTHORITY. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to di-isononylphthalate (DINP) for use in food contact materials, Question N°EFSA-Q-2003-194, EFSA Journal 244, 1-10

EFSA 2005d, EUROPEAN FOOD SAFETY AUTHORITY. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC)on a request from the commission related to di-isodecylphthalate (DIDP) for use in food contact materials, EFSA Journal 245, 1–14.

EFSA 2005e, EUROPEAN FOOD SAFETY AUTHORITY. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request frommthe commission related to bis(2-ethylhexyl)phthalate (DEHP) for use in food contact materials, Question N°EFSA-Q-2003-191, EFSA Journal 243, 1–20.

EFSA 2005d, EUROPEAN FOOD SAFETY AUTHORITY. Opinion of the scientific comitte on a request from EFSA related a harmonised approach for risk assesmnet of substances and carcinogenic, EFSA-Q-2004-020, EFSA Journal 282,1-31.

EFSA 2009, EUROPEAN FOOD SAFETY AUTHORITY .Statement on the presence of 4-methylbenzophenone found in breakfast cereals. EFSA J. RN-243:1–19. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812121190260462 3.html. acc [05/05/2017].

EFSA 2010, EUROPEAN FOOD SAFETY AUTHORITY. Management of left-censored data in dietary exposure assessment of chemical substances. EFSA Journal ,18-22.

EFSA 2013, EUROPEAN FOOD SAFETY AUTHORITY..International Frameworks dealing with human risk assessment on combined exposure to multiple chemicals. EFSA J. 11 (7), 3313. http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812121190260464 5.html. acc [01/05/2017].

EFSA 2015, EUROPEAN FOOD SAFETY AUTHORITY. Scientific opinion on Bisphenol A. http://www.efsa.europa.eu/sites/default/files/corporate_publications/files/factsheetbpa150121.pdf acc [01/05/2018]

EFSA SO 2015, EUROPEAN FOOD SAFETY AUTHORITY. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs. EFSA Journal, 13(1), 3978.

EPCCP 2009. EUROPEAN PARLIAMENT AND OF THE COUNCIL ON COSMETIC PRODUCTS. Commission Regulation (EU) Nº 358/2014 of 9 April 2014 amending Annexes II and V to Regulation (EC) Nº 1223/2009 of the European Parliament and of the Council on cosmetic products. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/uri=CELEX:32014R0358&from=RO (acc08/01/2018)

ESPACHS-BARROSO, A., SOLIVA-FORTUNY, R.C. and MARTÍN-BELLOSO, O., 2005. A natural clouding agent from orange peels obtained using polygalacturonase and cellulase. Food Chemistry 92, 55–61

ESTEBAN, M. and CASTAÑO, A., 2009. Non-invasive matrices in human biomonitoring: A review. Environment International 35(2),438-49.

ETSBEP 2015. EXPERT TEAM TO SUPORT BIOMONITORING IN EUROPE. Expert Team to Suport Biomonitoring in Europe protocol for sample collection in biomonitoring programs in Europe. Avaiable at : http:// www.eu-humanbiomonitoring.org/sub/esbio.htm. Acceso (15.01.2015)

ETZEL, T.M., CALAFAT, A.M., YE, X., CHEN, A., LANPHEAR, B.P., SAVITZ, D.A., YOLTON, K. and BRAUN, J.M., 2017. Urinary triclosan concentrations during pregnancy and birth outcomes. Environmental Research 156, 505-511.

EU 2003. EUROPEAN COMMISION. European Union Risk Assessment Report (EU RAR): Bisphenol-A Einecs-No. 201-245-8, 1-344.

EU 2005. EUROPEAN COMMMISSION REGULATION 1895/2005 epoxy derivates. Commission Regulation (EC) No 1895/2005 of 18 November 2005 on the restriction of use of certain epoxy derivatives in materials and articles intended to come into contact with food. ELI: http://data.europa.eu/eli/reg/2005/1895/oj acc [18-01-2018]

EU 2007. EUROPEAN UNION, COMMISSION DIRECTIVE 2007/19/EC. Amending Directive 2002/72/EC relating to plastic materials and articles intended to come into contact with food and Council Directive 85/572/EEC laying down the list of simulants to be used for testing migration of constituents of plastic materials and articles intended to come into contact with foodstuffs, Off. J. Eur. Commun. L. 91 (2007) 17–36.

EU 2008. EUROPEAN COMMMISSION REGULATION. Regulation (EC) No 1272/2008 of the European Parliament and the Council of 16 December 2008 on classification , labelling and packaging of substances and mixtures, amending and repealing directives 67/548/EEC

and 1999/45/EC, and amending regulation (EC) No 1907/2006.Off J. Eur. Union 51,1-219 (31 December, L 353)

EU 2009. EUROPEAN PARLIANMENT. Regulation (EC) Nº 1223/2009 of the European Parlianment and of the Council of 30 November 2009 on cosmetic products. http://eur-lex.europa.eu/legal- content/EN/TXT/PDF/?uri=CELEX:32009R1223&from=EN (acc: 08/01/2018)

EUR 24815EN 2011. Technical Guidelines on Testing the Migration of Primary Aromatic Aminesfrom Polyamide Kitchenware and of Formaldehyde from Melamine Kitchen-ware.

EU 10/2011. EUROPEAN COMMMISSION REGULATION. European commission Regulation (EU) No. 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, Off.J.Eur. Union L12(2011)1.

EU 284/2011. EUROPEAN COMMMISSION REGULATION. Commission Regulation (EU) No 284/2011 of 22 March 2011 laying down specific conditions and detailed procedures for the import of polyamide and melamine plastic kitchenware originating in or consigned from the People's Republic of China and Hong Kong Special Administrative Region, China Off.J.Eur. Union L (2011)1

EUPIA 2013. EUROPEAN PRINTING INK ASSOCIATION. Inventory list comprising packaging ink raw materials applied to the non-food contact surface of food packaging, 2013.

EUR 25112 2017. EUROPEAN COMMMISSION.Guidance document on fat reduction factor, functional barrier concept, phthalates and primary aromatic amines, EUR 25112 EN JRC68007, http://publications.jrc.ec.europa.eu/repository/handle/JRC68007, [acc 10/6/2017].
Expert Team to Support Biomonitoring in Europe protocol for sample collection in biomonitoring programs in Europe. 2017. http://www.eu-humanbiomonitoring.org/sub/esbio.htm. [acc 20/6/2018].

EYERER, P., 2010. Plastics: Classification, Characterization, and Economic. Polymers - Opportunities and Risks I ,19-46.

FAN, Y., LIU, S. and XIE, Q., 2014. Rapid determination of phthalate esters in alcoholic beverages by conventional ionic liquid dispersive liquid–liquid microextraction coupled with high performance liquid chromatography. Talanta 119, 291-8.

FARAHANI, H., GANJALI, M.R., DINARVAND, R. and NOROUZI, P., 2008. Screening method for phthalate esters in water using liquid-phase microextraction based on the solidification of a floating organic microdrop combined with gas chromatography–mass spectrometry. Talanta 76(4), 718-23.

FASANO, E., BONO-BLAY, F., CIRILLO, T., MONTUORI, P. and LACORTE, S., 2012. Migration of phthalates, alkylphenols, bisphenol A and di(2-ethylhexyl)adipate from food packaging. Food Control 27,132-138

FAVARO PEREZ M., PADULAM., MOITINHO D., BEATRIZ C., BOTTOLI G., 2019.Primary aromatic amines in kitchenware: Determination by Liquid chromatography -tandem mass spectrometry. Journal of chromatography A (in press).

FIERENS, T., SERVAES, K., VAN HOLDERBEKE, M., GEERTS, L., DE HENAUW, S., SIOEN, I. and VANERMEN, G., 2012. Analysis of phthalates in food products and packaging materials sold on the Belgian market. Food Chemical and Toxicology 50(7), 2575-83.

FRANCESCA, I., PATRIZIA, P., LUCA, C., FEDERICO, M. and ANNALISA, R., 2015. Analysis of volatile compounds in powdered milk for infant nutrition by direct desorption (CIS4–TDU) and GC–MS. Talanta 141,195-9.

FRÖLICH 2014 A European plastics market and trend study. Life cycle analyses from virgin material until post-consumer waste scenarios. EcoSphere. Available at: http://ptfplus.com/onewebmedia/A%20European%20Plastics%20Market%20and%20Trend%20Study.pdf.

FROMME, H., BOLTE, G., KOCH, H.M., ANGERER, J., BOEHMER, S., DREXLER, H., MAYER, R. and LIEBL, B., 2007. Occurrence and daily variation of phthalate metabolites in the urine of an adult population. International Journal of Hygiene and Environmental Health 210(1), 21-33.

FROMME, H., GRUBER, L., SCHLUMMER, M., WOLZ, G., BÖHMER, S., ANGERER, J., MAYER, R., LIEBL, B. and BOLTE, G., 2007. Intake of phthalates and di(2-ethylhexyl)adipate: Results of the Integrated Exposure Assessment Survey based on duplicate diet samples and biomonitoring data. Environmnt International. 33(8),1012-20.

GALLART-AYALA, H., MOYANO, E. and GALCERAN, M.T., 2011a. Analysis of bisphenols in soft drinks by on-line solid phase extraction fast liquid chromatography-tandem mass spectrometry. Analytica Chimica Acta 683(2), 227-233.

GALLART-AYALA, H., MOYANO, E. and GALCERAN, M.T., 2011b. Fast liquid chromatography-tandem mass spectrometry for the analysis of bisphenol A-diglycidyl ether, bisphenol F-diglycidyl ether and their derivatives in canned food and beverages. Journal of Chromatography A, 1218(12),1603-1610.

GALLART-AYALA, H., NÚÑEZ, O. and LUCCI, P., 2013. Recent advances in LC-MS analysis of food-packaging contaminants. Trends in Analytical Chemistry 42, 99-124

GALLART-AYALA, H., NÚÑEZ, O., MOYANO, E. and GALCERAN, M.T., 2011. Analysis of UV ink photoinitiators in packaged food by fast liquid chromatography at sub-ambient temperature coupled to tandem mass spectrometry. Journal of Chromatography A 1218(3), 459-66

GALLO F., FOSSI C., WEBER R., SANTILLO D., SOUSA J., INGRAM I., NADAL A., ROMANO D., 2018. Marine litter plastics and microplastics and therir toxic chemicals components: the need for urgent preventive measures. Environmental Science Europe 30,13.

GAO, L., ZOU, J., LIU, H., ZENG, J., WANG, Y. and CHEN, X., 2013. Determination of bisphenol A in thermal printing papers treated by alkaline aqueous solution using the combination of single-drop microextraction and HPLC. Journal of Separation Science, 36(7) 1298-1303.

GARCÍA V., SENDOM R., BUSTOS J., PASEIRO P., RODRÍGUEZ A., 2019. Estimates of dietary exposure of Spanish population to packaging contaminants from cereal based foods contained in plastic materials. Food and Chemical Toxicology 128, 180-192.

GARCÍA LAVANDEIRA, J., SALGADO-PETINAL, C., BLANCO, E. and CELA, R., 2010. A sensitive and efficient procedure for the high throughput determination of banned aromatic amines in textiles and leather products aided by advanced sample composition. Analytical and Bioanalytical Chemistry, 397(2)751-763.

GEENS, T., AERTS, D., BERTHOT, C., BOURGUIGNON, J., GOEYENS, L., LECOMTE, P., MAGHUIN-ROGISTER, G., PIRONNET, A., PUSSEMIER, L., SCIPPO, M., VAN LOCO, J. and COVACI, A., 2012. A review of dietary and non-dietary exposure to bisphenol-A. Food and Chemical Toxicology 50(10), 3725-40

GEENS, T., BRUCKERS, L., COVACI, A., SCHOETERS, G., FIERENS, T., SIOEN, I., VANERMEN, G., BAEYENS, W., MORRENS, B., LOOTS, I., NELEN, V., DE BELLEVAUX, B.N., LAREBEKE, N.V. and HOND, E.D., 2014. Determinants of bisphenol A and phthalate metabolites in urine of Flemish adolescents. Environmental Research134,110-7

GEENS, T., GOEYENS, L. and COVACI, A., 2011. Are potential sources for human exposure to bisphenol-A overlooked. International Journal of Hygiene and Environmental Health 214(5), 339-47

GEYER R., JAMBECK J.R, LAW K., 2017, Production, use, and fate of all plastics ever made. Science Advances, 3, 170078.

GROH K., BACKHAUS T., CARNEY-ALMROTH B., GEUEKE B., INOSTROZA P., LENNQIST A., LESLIE H., MAFFINI M., SLUNGE D., TRASANDE L., WARHURST M., MUNCKE J., 2019.Overview of known plastic packaging -associated chemicals and their hazards. Science of the Total Environment 651, 3253-3268.

GRUBER, H.F., 1992. Photoinitiators for free radical polymerization. Progress in Polymer Science, 17, 953-1044.

GB/T 21911-2008, Standard of the People's Republic of China, Determination of phthalate esters in foods. 8, 2008 http://www.gbstandards.org acc [01/02/2016].

HAYASAKA, Y., 2014. Analysis of phthalates in wine using liquid chromatography tandem mass spectrometry combined with a hold-back column: Chromatographic strategy to avoid the influence of pre-existing phthalate contamination in a liquid chromatography system. Journal of Chromatography A 1372C, 120-127

HBM4EU, 2017-last update, Deliverable Report D 4.2. WP 4 Prioritisation and input to the Annual Work Plan. Deadline: June, 2017. Upload by Coordinator: 28.06. 2017 https://www.hbm4eu.eu/ acc. [01/03/2018].

HE, J., LV, R., ZHU, J. and LU, K., 2010. Selective solid-phase extraction of dibutyl phthalate from soybean milk using molecular imprinted polymers. Analalytical Chimica Acta. 661(2), 215-21.

HEFFERNAN, A.L., THOMPSON, K., EAGLESHAM, G., VIJAYASARATHY, S., MUELLER, J.F., SLY, P.D. and GOMEZ, M.J., 2016. Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine. Talanta 151, 224-233.

HEIDBRER L, BABLOK I., DREWS S., MENZEL C., 2019. Tackling the plastic problem: A review on perceptions, behaviors and interventions Science of Total Environment 668, 1077-1093.

HINES EP., MENDOLA P., VON EHRENSTEIN OS, YE X, CALAFAT AM. FENTON SE, 2015. Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women. Reproductive Toxicology 54,120-8.

HRÁDKOVÁ, P., POUSTKA, J., HLOUŠKOVÁ, V., PULKRABOVÁ, J., TOMANIOVÁ, M. and HAJŠLOVÁ, J., 2010. Perfluorinated compounds: Occurrence of emerging food contaminants in canned fish and seafood products. Czech Journal of Food Sciences, 28(4), 333-342.

HUANG, Z., PAN, X., WU, P., CHEN, Q., HAN, J. and SHEN, X., 2013. Validation (in-house and Collaboratory) of the quantification method for ethyl carbamate in alcoholic beverages and soy sauce by GC–MS. Food Chemistry 141(4), 4161-5

HUANG R., LIU Z., YUAN S., YIN H., DANG Z., WU P., 2017.Worldwide human daily intakes of bisphenol A (BPA) estimated from global urinary concentration data (2000-2016) and its risk analysis. Environmental Pollution 230, 143-152.

IARC 2016. International Agency for Research on Cancer (IARC). Monographs on the Evaluation of the carcinogenic risk of chemicals to humans, http://monographs.iarc.fr/ENG/Classification/index.php,2016 acc [03-05-16, 2016].

INSTT 2011, INSTITUTO NACIONAL DE SEGURIDAD E HIGIENE EN EL TRABAJO, Documentación toxicológica para el establecimiento del límite de exposición profesional de bisfenol A, http://www.insht.es/InshtWeb/Contenidos/Documentacion/LEP%20_VALORES%20LIMITE/Doc_Toxicologica/Ficheros%202011/DLEP%2060%20Bisfenol%20A.pdf accs [15/01/2019].

JARDIM C., MELO P., SOARES D., COSTA M., 2015. Determination of parabens in urine samples by microextraction using packed sorbent and ultra-performance liquid

chromatography coupled to tandem mass spectrometry. Journal of Chromatography B 974, 35-41.

J. ACEÑA, S. STAMPACHIACCHIERE, S. PÉREZ, D. BARCELÓ, 2015. Advances in liquid chromatography-high-resolution mass spectrometry for quantitative and qualitative environmental analysis. Analytical and Bioanalytical Chemistry 407, 6289-6299.

JIA, W., CHU, X., LING, Y., HUANG, J. and CHANG, J., 2014. Analysis of phthalates in milk and milk products by liquid chromatography coupled to quadrupole Orbitrap high-resolution mass spectrometry. Journal of Chromatography A 1362,110-8.

JIMÉNEZ-DÍAZ, I., ARTACHO-CORDÓN, F., VELA-SORIA, F., BELHASSEN, H., ARREBOLA, J.P., FERNÁNDEZ, M.F., GHALI, R., HEDHILI, A. and OLEA, N., 2016. Urinary levels of bisphenol A, benzophenones and parabens in Tunisian women: A pilot study. Science of the Total Environment 562, 81-88

JOGSTEN, I.E., PERELLÓ, G., LLEBARIA, X., BIGAS, E., MARTÍ-CID, R., KÄRRMAN, A. and DOMINGO, J.L., 2009. Exposure to perfluorinated compounds in Catalonia, Spain, through consumption of various raw and cooked foodstuffs, including packaged food. Food and Chemical Toxicology, 47(7) 1577-1583.

JOSÉ BARTUAL SÁNCHEZ, NTP 108: Criterios toxicológicos generales para los contaminantes químicos. http://www.insht.es/InshtWeb/Contenidos/Documentacion/FichasTecnicas/NTP/Ficheros/101a200/ntp_108.pdf acc [15/12/18].

KANNAN, K., CORSOLINI, S., FALANDYSZ, J., FILLMANN, G., KUMAR, K.S., LOGANATHAN, B.G., MOHD, M.A., OLIVERO, J., WOUWE, N.V., YANG, J.H. and ALDOUS, K.M., 2004. Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. Environmental science & technology, 38(17) 4489-4495.

KATSIKANTAMIA I., COLOSIOC C., ALEGAKISB A., TZATZARAKISB M., VAKONAKIB E., RIZOSA A., DIMOSTHENIS A., SARIGIANNISD D., TSATSAKISB A., 2019. Estimation of daily intake and risk assessment of organophosphorus pesticides based on biomonitoring data – The internal exposure approach. Food and Chemical Toxicology 123, 57–71.

KAWAKAMI, T., ISAMA, K., NAKASHIMA, H., TSUCHIYA, T. and MATSUOKA, A., 2010. Analysis of primary aromatic amines originated from azo dyes in commercial textile products in Japan. Journal of Environmental Science and Health, Part A, 45(10) 1281-1295.

Khedr A, 2013. Optimized extraction method for LC–MS determination of bisphenol A, melamine and di(2-ethylhexyl) phthalate in selected soft drinks, syringes, and milk powder. J Chromatogr B Analytical Technologies on Biomedical and Life Science 930, 98-103.

KENNETH, M. and BUGUSU, B., 2007. Food packaging: Roles, materials, and environmental. Journal of Food Science 72(3), R39-55

KOUTSIMANIS, G., GETTER, K., BEHE, B., HARTE, J. and ALMENAR, E., 2012. Influences of packaging attributes on consumer purchase decisions for fresh produce. Appetite. 59(2), 270-80

KRISHNAN K., GAGNÉ M., NONG A., AYLWARD LL., HAYS SM., 2010. Biomonitoring Equivalents for bisphenol A (BPA). Regulatory Toxicology and Pharmacology. 58(1), 18-24.

LATINI, G., VERROTTI, A. and DE FELICE, C., 2004. DI-2-Ethylhexyl Phthalate and Endocrine Disruption: A Review. Curr Drug Targets Immune Endocr Metabol Disord. Reproductive Toxicology 4(1), 37-40

LEE, H., DÂEON, J. and MABURY, S.A., 2010. Biodegradation of Polyfluoroalkyl Phosphates as a Source of Perfluorinated Acids to the Environment. Environmental science & technology, 44(9), 3305-3310.

LEE J., CHOI K., PARK J., MOON H., CHOI G., LEE J., SUH E., KIM S, 2017. Bisphenol A distribution in serum, urine, placenta, breast milk, and umbilical cord serum in a birth panel of mother-neonate pairs. Science of the Total Environment 626,1494-1501

LIAO C and KANNAN K. 2014. A survey of bisphenol A and other bisphenol analogues in foodstuffs from nine cities in China. Food Additives and Contamaminants Part A 31(2), 319-29

LIAO, C., LIU, F., ALOMIRAH, H., LOI, VU DUC, MOHD, M.A., MOON, H., NAKATA, H., KANNAN, K., 2012. Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures. Environmental Science and Technology 46, 6860–6866.

LUTTER, P., SAVOY-PERROUD, M., CAMPOS-GIMENEZ, E., MEYER, L., GOLDMANN, T., BERTHOLET, M., MOTTIER, P., DESMARCHELIER, A., MONARD, F., PERRIN, C., ROBERT, F. and DELATOUR, T., 2011. Screening and confirmatory methods for the determination of melamine in cow's milk and milk-based powdered infant formula: Validation and proficiency-tests of ELISA, HPLC-UV, GC-MS and LC-MS/MS. Food Control 22, 903-913

MAGI, E., SCAPOLLA, C., DI CARRO, M. and LISCIO, C., 2010. Determination of endocrine-disrupting compounds in drinking waters by fast liquid chromatography-tandem mass spectrometry. Journal of Mass Spectrometry, 45(9),1003-1011.

MAKKLIANG, F., KANATHARANA, P., THAVARUNGKUL, P. and THAMMAKHET, C., 2015. Development of magnetic micro-solid phase extraction for analysis of phthalate esters in packaged food. Food Chemistry 166C, 275-282 ·

MALARVANNAN, G., ONGHENA, M., VERSTRAETE, S., VAN PUFFELEN, E., JACOBS, A., VANHOREBEEK, I., VERBRUGGEN, S.C.A.T., JOOSTEN, K.F.M., VAN DEN BERGHE, G., JORENS, P.G. and COVACI, A., 2019. Phthalate and alternative plasticizers in indwelling medical devices in pediatric intensive care units. Journal of hazardous materials 363, 64-72.

MALLOZZI, M., BORDI, G., GARO, C. and CASERTA, D., 2016. The effect of maternal exposure to endocrine disrupting chemicals on fetal and neonatal development: A review on the major concerns. Birth Defects Research Part C: Embryo Today: Reviews 108(3), 224-242.

MARÍN, S.,2004. Estudios de dieta total. Exposición de la población de la Comunidad Valenciana a metales y evaluación del riesgo.Tesis doctoral, 38-50.

MARÍN, S., PARDO, O., SÁNCHEZ, A., SANCHIS, Y., VÉLEZ, D., DEVESA, V., FONT, G. and YUSÀ, V., 2018. Assessment of metal levels in foodstuffs from the Region of Valencia (Spain). Toxicology Report 5, 654-670.

MARTIN, J.W., ELLIS, D.A., MABURY, S.A., HURLEY, M.D. and WALLINGTON, T.J., 2006. Atmospheric Chemistry of Perfluoroalkanesulfonamides: Kinetic and Product Studies of the OH Radical and Cl Atom Initiated Oxidation of N-Ethyl Perfluorobutanesulfonamide. Environmental science & technology 40(3),864-872.

MATTAROZZI, M., LAMBERTINI, F., SUMAN, M. and CARERI, M., 2013. Liquid chromatography–full scan-high resolution mass spectrometry-based method towards the comprehensive analysis of migration of primary aromatic amines from food packaging. Journal of Chromatography A. 1320, 96-102.

MATTISON, D.R., KARYAKINA, N., GOODMAN, M. and LAKIND, J.S., 2014. Pharmaco- and toxicokinetics of selected exogenous and endogenous estrogens: A review of the data and identification of knowledge gaps. Critical reviews in toxicology 44(8), 696-724.

MCCALL, E., KEEGAN, J. and FOLEY, B., 2012. Primary aromatic amine migration from polyamide kitchen utensils: method development and product testing. Food Additives & Contaminants: Part A 29(1), 149-160.

MEI, H., HSIEH, Y., NARDO, C., XU, X., WANG, S., NG, K. and KORFMACHER, W.A., 2003. Investigation of matrix effects in bioanalytical high-performance liquid chromatography/tandem mass spectrometric assays: application to drug discovery. Rapid Communications in Mass Spectrometry 17(1), 97-103.

MEZCUA, M., MARTÍNEZ-UROZ, M.A., GÓMEZ-RAMOS, M.M., GÓMEZ, M.J., NAVAS, J.M. and FERNÁNDEZ-ALBA, A.R., 2012. Analysis of synthetic endocrine-disrupting chemicals in food: A review. Talanta 100, 90-106.

MÍGUEZ, J., HERRERO, C., QUINTÁS, I., RODRÍGUEZ, C., GIGOSOS, P.G. and MARIZ, O.C., 2012. A LC–MS/MS method for the determination of BADGE-related and BFDGE-related compounds in canned fish food samples based on the formation of [M+NH4]+ aducts. Food Chemistry 135(3),1310-5.

MOORE, L. and POSTLE, M., 1994. Risk-benefit analysis and case study on tributyl tin.International biodeterodation and biodegradation 34, 401-412

MOOS, R.K., ANGERER, J., WITTSIEPE, J., WILHELM, M., BRÜNING, T. and KOCH, H.M., 2014. Rapid determination of nine parabens and seven other environmental phenols in urine samples of German children and adults. International Journal of Hygiene and Environmental Health 217(8), 845-53.

MOOS, R.K., KOCH, H.M., ANGERER, J., APEL, P., SCHRÖTER-KERMANI, C., BRÜNING, T. and KOLOSSA-GEHRING, M., 2015. Parabens in 24h urine samples of the German Environmental Specimen Bank from 1995 to 2012. International Journal of Hygiene and Environmental Health 218(7), 666-74

MOREIRA, M.A., ANDRÉ, L.C. and CARDEAL, Z.D.L., 2015. Analysis of plasticiser migration to meat roasted in plastic bags by SPME–GC/MS. Food Chemistry 178,195-200.

MORETA, C. and TENA, M.T., 2014. Determination of perfluorinated alkyl acids in corn, popcorn and popcorn bags before and after cooking by focused ultrasound solid–liquid extraction, liquid chromatography and quadrupole-time of flight mass spectrometry. Journal of Chromatography A. 1355, 211-8

MORTENSEN, G.K., MAIN, K.M., ANDERSSON, A.-., LEFFERS, H. and SKAKKEBÆK, N.E., 2005. Determination of phthalate monoesters in human milk, consumer milk, and infant formula by tandem mass spectrometry (LC-MS-MS). Analytical and Bioanalytical Chemistry 382(4), 1084-1092.

MUNCKE, J., MYERS, J.P., SCHERINGER, M. and PORTA, M., 2014. Food packaging and migration of food contact materials: will epidemiologists rise to the neotoxic challenge? Journal of epidemiology and community health 68(7), 592-620.

MYRIDAKIS, A., FTHENOU, E., BALASKA, E., VAKINTI, M., KOGEVINAS, M. and STEPHANOU, E.G., 2015. Phthalate esters, parabens and bisphenol-A exposure among mothers and their children in Greece (Rhea cohort). Environmental International 83, 1-10.

NASSAN, F.L., WILLIAMS, P.L., GASKINS, A.J., BRAUN, J.M., FORD, J.B., CALAFAT, A.M. and HAUSER, R., 2019. Correlation and temporal variability of urinary biomarkers of chemicals among couples: Implications for reproductive epidemiological studies. 123,181-188.

NAVIA P., D.P., AYALA A., A.A. and VILLADA C., H.S., 2014. Interacciones empaque-alimento: migración. Revista Ingenierias Universidad de MedellÃn, 13(25) 99-113.

NERÍN, C., 2016. Plastics and Polymers for Food Packaging Manufacturing. Reference Module in Food Science 10.1016/B978-0-08-100596-5.03195-4

NERIN, C., ALFARO, P., AZNAR, M. and DOMEÑO, C., 2013. The challenge of identifying non-intentionally added substances from food packaging materials: A review. Analytical Chimica Acta 775, 14-24

NING, L., FU-PING, Z., HAI-TAO, C., SI-YUAN, L., CHEN, G., ZHEN-YANG, S. and BAO-GUO, S., 2011. Identification of volatile components in Chinese Sinkiang fermented camel milk using SAFE, SDE, and HS-SPME-GC/MS. Food Chemistry 129(3),1242-52

NÚÑEZ, O., GALLART-AYALA, H., MARTINS, C.P.B. and LUCCI, P., 2012. New trends in fast liquid chromatography for food and environmental analysis. Journal of Chromatography A 1228, 298-323.

NSOPC 2011. National Standards of the People's Republic of China, Standards for use of food addi-tives. GB 2760-2011.

OSTROVSKÝ, I., ČABALA, R., KUBINEC, R., GÓROVÁ, R., BLAŠKO, J., KUBINCOVÁ, J., ŘIMNÁČOVÁ, L. and LORENZ, W., 2011. Determination of phthalate sum in fatty food by gas chromatography. Food Chemistry 124, 392–395

PAPKOV, S.P., 1982. Classification of polymers according to their phase state. Polymer Science 8, 1934-1939.

PAWLISZYN, J., 2012. 2 - Theory of Solid-Phase Microextraction. Oxford: Elsevier.

PARK S., PARK S., JEONG M., CHOI J., KIM M.,2018. Fast and simple determination and exposure assessment of bisphenol A, and diphenylcarbonate transferred from poly-carbonate food-contact materials to food simulants. Chemosphere 203,300-306.

PLASTICSEUROPE 2016, The Facts-2016 https://www.plasticseurope.org/application/files/4315/1310/4805/plastic-the-fact-2016.pdf

PLASTICSEUROPE, 2018. Plastics-the Facts 2018: An analysis of European Plastics Production. Demand and Wate Data, Brussels, Belgium. https://www.plasticseurope.org/application/files/4315/1310/4805/plastic-the-fact-2018.pdf

PENG, F., JI, W., ZHU, F., PENG, D., YANG, M., LIU, R., PU, Y. and YIN, L., 2016. A study on phthalate metabolites, bisphenol A and nonylphenol in the urine of Chinese women with unexplained recurrent spontaneous abortion. Environmental Research 150, 622-628.

PÉREZ, R., DOMENECH, E., COSCOLLÀ, C. and YUSÀ, V., 2017. Human Biomonitoring of food contaminants in Spanish children: Design, sampling and lessons learned. International Journal of Hygiene and Environmental Health (8), 1242-1251

PÉREZ, R., SUELVES, T., MOLINA, Y., CORPAS-BURGOS, F. and YUSÀ, V., 2019. Biomonitoring of mercury in hair of children living in the Valencian Region (Spain). Exposure and risk assessment. Chemosphere 217, 558-566.

PÉREZ-PALACIOS, D., FERNÁNDEZ-RECIO, M.Á, MORETA, C. and TENA, M.T., 2012 Determination of bisphenol-type endocrine disrupting compounds in food-contact recycled-paper materials by focused ultrasonic solid–liquid extraction and ultra performance liquid chromatography-high resolution mass spectrometry. Talanta.99,167-74.

PEZO, D., FEDELI, M., BOSETTI, O. and NERÍN, C., 2012. Aromatic amines from polyurethane adhesives in food packaging: The challenge of identification and pattern recognition using Quadrupole-Time of Flight-Mass Spectrometry. Analytical Chimica Acta 756, 49-59.

PHILIPS E., JADDOE V., ASIMAKOPOULOS A., KANNAN K., STEEGERS E., SANTOS S. and TRASANDE L, 2018. Bisphenol and phthalate concentrations and its determinants among pregnant women in a population-based cohort in the Netherlands, 2004–5. Environmnetal Research161, 562-572.

PICÓ, Y., 2013. Ultrasound-assisted extraction for food and environmental samples. Trends in Analytical Chemistry 43, 84-99.

PICO, Y., FARRÃ©, M., LLORCA, M. and BARCELO, D., 2011. Perfluorinated Compounds in Food: A Global Perspective. Critical Review on Food Science and Nutrition (7), 605-25.

POLLACK A., PERKINS J., SJAARDA L., MUMFORD S., KANNAN K., PHILIPPAT C, WACTAWSKI-WENDE J.and SCHISTERMAN E.,2016. Variability and exposure classification of urinary phenol and paraben metabolite concentrations in reproductive-aged women. Environmnetal Research 151, 513-520.

POSE-JUAN, E., FERNÁNDEZ-CRUZ, T. and SIMAL-GÁNDARA, J., 2016. State of the art on public risk assessment of combined human exposure to multiple chemical contaminants. Trends in Food Science &Technology 55,11-28

PREVEDOUROS, K., COUSINS, I.T., BUCK, R.C. and KORZENIOWSKI, S.H., 2006. Sources, Fate and Transport of Perfluorocarboxylates. Environmental science & technology, 40(1), 32-44.

QUIRÓS-ALCALÁ L., BUCKLEY J.,BOYLE M, Parabens and measures of adiposity among adults and children from the U.S. general population: NHANES 2007–2014. International Journal of Hygiene and Environmental Health 4, 221-8.

RAYMER, J.H., MICHAEL, L.C., STUDABAKER, W.B., OLSEN, G.W., SLOAN, C.S., WILCOSKY, T. and WALMER, D.K., 2012. Concentrations of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) and their associations with human semen quality measurements. Reproductive and Toxicology 33(4), 419-27.

RASSF 2005.THE RAPID ALERT SYSTEM FOR FOOD AND FEED. Annual Report. https://ec.europa.eu/food/sites/food/files/safety/docs/rasff_annual_report_2005_en.pdf acc. [07/01/2017].

RASFF 2019. European Comission, Notifications list https://webgate.ec.europa.eu/rasffwindow/portal/?event=notificationsList&StartRow=101[acc 20/6/2016].

RE 1935/2004. Reglamento del Parlamento Europeo y del Consejo, de 27 de octubre de 2004, sobre los materiales y objetos destinados a entrar en contacto con alimentos y por el que se

derogan las Directivas 80/590/CEE y 89/109/CEE. https://www.boe.es/buscar/doc.php?id=DOUE-L-2004-82656 [acc 20/6/2016].

RIOS J., MORALES A. MÁRQUEZ-RUIZ G., 2010. Headspace solid-phase microextraction of oil matrices heated at high temperature and phtalate esters determination by gas chromatography multistage mass spectrometry. Talanta 80(5), 2076-82.

ROCA, M., SÁNCHEZ, A., PÉREZ, R., PARDO, O. and YUSÀ, V., 2016. Biomonitoring of 20 elements in urine of children. Levels and predictors of exposure. Chemosphere 144:1698–705

ROCHA, B.A., DE OLIVEIRA, A.R.M. and BARBOSA, F., 2018. A fast and simple air-assisted liquid-liquid microextraction procedure for the simultaneous determination of bisphenols, parabens, benzophenones, triclosan, and triclocarban in human urine by liquid chromatography-tandem mass spectrometry. Talanta138, 94-101.

ROCHESTER, J.R. and BOLDEN, A.L., 2015. Bisphenol S and F: A Systematic Review and Comparison of the Hormonal Activity of Bisphenol A Substitutes. Environmental health perspectives 123(7), 643-650.

SAKHI A, SABAREDZOVIC E., PAPADOPOULOU E, CEQUIER E., THOMSEN C., 2018. Levels, variability and determinants of environmental phenols in pairs of Norwegian mothers and children. Environment International 114, 242-251.

SADANORI KONISHI, G.K., 2008. Information Criteria and Statistical Modeling, Springer 9, 112-259.

SAGRATINI, G., CAPRIOLI, G., CRISTALLI, G., GIARDINÁ, D., RICCIUTELLI, M., VOLPINI, R., ZUO, Y. and VITTORI, S., 2008. Determination of ink photoinitiators in packaged beverages by gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry. Journal of Chromatography A 1194(2), 213-20

SANCHIS, Y., COSCOLLÀ , C. and YUSA, V., 2018. Comprehensive analysis of Photoinitiators and Primary Aromatic Amines in food contact materials using Liquid Chromatography High-Resolution Mass Spectrometry. Talanta 191, 111-118.

SANCHIS, Y., COSCOLLÀ, C., ROCA, M. and YUSÀ, V., 2015. Target analysis of primary aromatic amines combined with a comprehensive screening of migrating substances in kitchen utensils by liquid chromatography–high resolution mass spectrometry. Talanta. 138, 290-297

SANCHIS, Y., YUSÀ, V. and COSCOLLÀ, C., 2017. Analytical strategies for organic food packaging contaminants. Journal of Chromatography A 1490, 22-46

SANTE/11813/2017. European Commission Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed.

SATOH, K., OHYAMA, K., AOKI, N., IIDA, M. and NAGAI, F., 2004. Study on anti-androgenic effects of bisphenol a diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE) and their derivatives using cells stably transfected with human androgen receptor, AR-EcoScreen. Food Chemistry and Toxicology. 42(6), 983-93

SCHUBERT, J., KAPPENSTEIN, O., LUCH, A. and SCHULZ, T.G., 2011. Analysis of primary aromatic amines in the mainstream waterpipe smoke using liquid chromatography–electrospray ionization tandem mass spectrometry. Journal of Chromatography A. 1218(33), 5628-37.

SCHLUMPF M., KYPKE K., WITTASSEK M., ANGERER J., MASCHER H., MASCHER D., VÖKT C., BIIRCHLER M., LICHTENSTEIGER W., 2010. Exposure patterns of UV filters, fragances, parabens, phthalates, organochlor pesticides, PBDEs, and PCBs in human milk: Correlation of UV filters with use of cosmetics. Chemosphere 81, 1171-1183.

SHEN, D., LIAN, H.-., DING, T., XU, J.-. and SHEN, C.-., 2009. Determination of low-level ink photoinitiator residues in packaged milk by solid-phase extraction and LC-ESI/MS/MS using triple-quadrupole mass analyzer. Analytical and Bioanalytical Chemistry 395(7), 2359-2370.

SINCLAIR, E., KIM, S.K., AKINLEYE, H.B. and KANNAN, K., 2007. Quantitation of Gas-Phase Perfluoroalkyl Surfactants and Fluorotelomer Alcohols Released from Nonstick Cookware and Microwave Popcorn Bags. Environmental science & technology, 41(4), 1180-1185.

SIRACUSA J., YIN L., MEASEL E., LIANG S., YU X., 2018. Effects of bisphenol A and its analogs on reproductive health: A mini review. Reproductive Toxicology 79, 96-123.

STAPLS, C.A., DOME, P.B., KLECKA, G.M., OBLOCK, S.T. and HARRIS, L.R., 1998. A review of the environmental fate, effects, and exposures of bisphenol. Chemosphere 36(10), 2149-73.

STATISTA, 2018. Ranking of the leading countries of plastics consumption in Europe in 2015. Available at: https://www.statista.com/statistics/756517/leading- countries-plastics-consumption-europe/. (Accessed 31/05/2018).

SUCIU, N.A., TIBERTO, F., VASILEIADIS, S., LAMASTRA, L. and TREVISAN, M., 2013. Recycled paper–paperboard for food contact materials: Contaminants suspected and migration into foods and food simulant. Food Chemistry 141(4), 4146-51

TAO, L., MA, J., KUNISUE, T., LIBELO, E.L., TANABE, S. and KANNAN, K., 2008. Perfluorinated Compounds in Human Breast Milk from Several Asian Countries, and in Infant Formula and Dairy Milk from the United States. Environmental science & technology, 42(22) 8597-8602.

CODEX STAN 1995. The Codex Committee on Food Additives and Contaminants, General standard for food additives. CODEX STAN 192-1995.

TRIER, X., OKHOLM, B., FOVERSKOV, A., BINDERUP, M. and PETERSEN, J.H., 2010. Primary aromatic amines (PAAs) in black nylon and other food-contact materials, 2004-2009. 27(9), 1325-35

UNEP 2009. Governments Unite to Step-up Reduction on Global DDT Reliance and Add Nine New Chemicals under International Treaty. http://chm.pops.int/Convention/Pressrelease/COP4Geneva8May2009/tabid/542/language/en-US/Default.aspx,2009 acc [09.03.15, 2015].

UNEP 2018. Overview Report I: Worldwide Initiatives to Identify Endocrine Disrupting Chemicals (EDCs) and Potential EDCs. International Panel on Chemical Pollution (IPCP).

VALVIA D., MONFORT N.,VENTURA R., CASASA,M, CASAS L., SUNYERA,J., VRIJHEID M., 2015. Variability and predictors of urinary phthalate metabolites in Spanish pregnant women. International Journl of Hygiene and Environmental Health 218, 220–231

VANDENBERG, L.N., HAUSER, R., MARCUS, M., OLEA, N. and WELSHONS, W.V., 2007. Human exposure to bisphenol A (BPA). Reproductive Toxicology 24(2),139-77.

VANDENTORREN, S., ZEMAN, F., MORIN, L., SARTER, H., BIDONDO, M., OLEKO, A. and LERIDON, H., 2011. Bisphenol-A and phthalates contamination of urine samples by catheters in the Elfe pilot study: Implications for large-scale biomonitoring studies. Environmental Research 111(6),761-4

VAVROUŠ, A., VÁPENKA, L., SOSNOVCOVÁ, J., KEJLOVÁ, K., VRBÍK, K. and JÍROVÁ, D., 2016. Method for analysis of 68 organic contaminants in food contact paper using gas and liquid chromatography coupled with tandem mass spectrometry. Food Control 60, 221-229

VAZQUEZ-ROIG, P. and PICÓ, Y., 2015. Pressurized liquid extraction of organic contaminants in environmental and food samples. Trends in analytical chemistry 71,55-64.

VENISSE, N., GRIGNON, C., BRUNET, B., THÉVENOT, S., BACLE, A., MIGEOT, V. and DUPUIS, A., 2014. Reliable quantification of bisphenol A and its chlorinated derivatives in human urine using UPLC–MS/MS method. Talanta 125, 284-92.

VERA, P., CANELLAS, E. and NERÍN, C., 2014. Migration of odorous compounds from adhesives used in market samples of food packaging materials by chromatography olfactometry and mass spectrometry (GC–O–MS). Food Chemistry 145, 237-44.

VERA, P., CANELLAS, E. and NERÍN, C., 2018. Identification of non volatile migrant compounds and NIAS in polypropylene films used as food packaging characterized by UPLC-MS/ QTOF. Talanta 188, 750-762.

W NELSON, Jessica, E HATCH, Elizabeth and WEBSTER, T., 2010. Exposure to Polyfluoroalkyl Chemicals and Cholesterol, Body Weight, and Insulin Resistance in the General U.S. Population. Environ Health Perspectives 118(2), 197-202

WANG, J., SHI, Y., PAN, Y. and CAI, Y., 2010. Perfluorinated compounds in milk, milk powder and yoghurt purchased from markets in China. Chinese Science Bulletin, 55(11), 1020-1025.

WHO 2006. WORLD HEALTH ORGANIZATION. The World Health Report 2006 - working together for health, https://www.who.int/whr/2006/en/ acc [09/01/19].

WHO 2002. WORLD HEALTH ORGANIZATION. A global strategy for food safety: safer food for better health, Geneva.http://www.who.int/ foodsafety/publications/genera/global_strategy/en acc [01/09/2018]

WHO 2010. WORLD HEALTH ORGANIZATION. Human biomonitoring, Facts and Figures, Copenhagen. http://www.euro.who.int/data/assets/pdf_file/0020/276311/Human-biomonitoring-facts-figures-en.pdf acc [01/09/2018].

WHO 2019. WORLD HEALTH ORGANIZATION. Food safety is everyone's bussines,. https://www.who.int/news-room/detail/06-06-2019-food-safety-is-everyones-business acc [01/06/2019]

WU, L., ZHANG, X., WANG, F., GAO, C., CHEN, D., PALUMBO, J.R., GUO, Y. and ZENG, E.Y., 2018. Occurrence of bisphenol S in the environment and implications for human exposure: A short review. Science of the Total Environment 615, 87-98.

WYPYCH, G., 2012. Handbook of Polymers 2nd Edition, ISBN: 9781927885116, Oxford: Elsevier, 153-198.

XU, D., DENG, X., FANG, E., ZHENG, X., ZHOU, Y., LIN, L., CHEN, L., WU, M. and HUANG, Z., 2014. Determination of 23 phthalic acid esters in food by liquid chromatography tandem mass spectrometry. Journal of Chromatography A 1324,49-56.

Xu Liang Cao 2010. Phthalate Esters in Foods: Sources, Occurrence, and Analytical Methods.Comprenhensive reviews on food science and food safety 9, 2-24.

YAN, H., CHENG, X. and LIU, B., 2011. Simultaneous determination of six phthalate esters in bottled milks using ultrasound-assisted dispersive liquid–liquid microextraction coupled with gas chromatography. Journal of Chromatography B Analytical Technologies and Biomedical Life Science 879(25), 2507-12

YANG, J., LI, Y., WANG, Y., RUAN, J., ZHANG, J. and SUN, C., 2015. Recent advances in analysis of phthalate esters in foods. 72, 81-97.

YANG, Y., GUAN, J., YIN, J., SHAO, B. and LI, H., 2014. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China. Chemosphere 112, 481-6

YUSA, V., MILLET, M., COSCOLLA, C. and ROCA, M., 2015. Analytical methods for human biomonitoring of pesticides. A review. Analyltical Chimica Acta 891, 15-31.

YUSA, V., YE, X. and CALAFAT, A.M., 2012. Methods for the determination of biomarkers of exposure to emerging pollutants in human specimens. Trends in Analytical Chemistry 38, 129-142

ZAFEIRAKI, E., COSTOPOULOU, D., VASSILIADOU, I., LEONDIADIS, L., DASSENAKIS, E., HOOGENBOOM, R.L.A.P. and VAN LEEUWEN, S.P.J., 2016. Perfluoroalkylated substances (PFASs) in home and commercially produced chicken eggs from the Netherlands and Greece. Chemosphere 144, 2106-12

ZAFEIRAKI, E., D COSTOPOULOU, Danae, VASSILIADOU, I., BAKEAS, E. and LEONDIADIS, L., 2014. Determination of perfluorinated compounds (PFCs) in various foodstuff packaging materials used in the Greek market. Chemosphere 94, 169-76.

ZHANG, S., ZHANG, Y., JI, G., XU, H., LIU, J. and SHI, L., 2016. Determination of Bisphenol A, Tetrabromobisphenol A and 4-Tert-Octylphenol in Children and Adults Urine Using High Performance Liquid Chromatography-Tandem Mass Spectrometry. Chinese Journal in Analytical Chemistry, 44, 19-24.

ZHOU J., CHENA X., PANA S, WANG J., ZHENG J., XUC J., ZHAO Y., CAIC Z., JINA M., 2019. Contamination status of bisphenol A and its analogues (bisphenol S ,F and B) in food stuffs and the implications for dietary exposure on adult residents in Zhejiang Province. Food Chemistry 294, 160-170.

ZHOU, X., KRAMER, J.P., CALAFAT, A.M., YE, X., 2014. Automated on-line column-switching high-performance liquid chromatography isotope dilution tandem mass spectrometry method for the quantification of bisphenol A, bisphenol F, bisphenol S, and 11 other phenols in urine. Journal of Chromatography B 944, 152–156.

ZHAO, H., LI, J., MA, X., HUO, W., XU, S. and CAI, Z., 2018. Simultaneous determination of bisphenols, benzophenones and parabens in human urine by using UHPLC-TQMS. Chinese Chemical Letters 29, 102-109.

ZHU, L., ZHU, T., MA, Y., NI, Y., WANG, Y. and YAN, Q., 2013. Rapid Determination of 15 Kinds of Phthalate Esters in Vegetable Juices by Hollow Fiber-Liquid Phase Microextraction Coupled with Gas Chromatography-Mass Spectrometr.y. Chinese Journal of Analytical Chemistry 41(7),1019-1120.

Anexos

Anexo I

ANEXOS: Información Suplementaria

Anexo I. Información suplementaria del articulo científico 1.

Editorial on “Analytical strategies for organic packaging contaminants” by Yovana Sanchis, Vicent Yusà and Clara Coscollà

Journal of Chromatography A, 1490 (2017) 21

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Editorial

Editorial on “Analytical strategies for organic packaging contaminants” by Yovana Sanchis, Vicent Yusà and Clara Coscollà

Foods can be contaminated in several ways: microorganisms, toxic metals, pesticides, environmental contaminants, and chemicals that migrate into the food from food containers (and other food contact materials). The review by Sanchis, Yusa, and Coscolla focuses on the chemical analysis applied to the latter problem. While food contact materials (FCMs) are officially classified into 17 groups by one regulation, this review gives most attention to plastics, and secondarily to paper and paper board wrappings, since these are the major FCMs.

The review is comprehensive, and divided into three substantial parts. Part 1 introduces and overviews the subject, including general types of FCMs and analytical regulation in the field. We learn that most of the analytical work has focused on testing the container materials *per se* rather than the foods, or conducting experiments with food stimulants (an artificial food such as aqueous acetic acid to simulate a beverage, or a synthetic adsorbent to simulate a dry food). Migration of chemicals into a food stimulant instead of an actual food makes the chemical analysis easier.

Part 2 discusses the major classes of food-container and food contact chemicals that have been studied with information about their origins, toxicity, and specific regulations: primary aromatic amines, bisphenols, perfluorinated compounds, phthalates, non-intentionally added substances (such as contaminants in raw materials), printing ink-photoinitiators, additives (e.g. antioxidants, stabilizers and plasticizers beyond phthalates), and mineral oil components.

Part 3 presents the main analytical methodologies, namely GC–MS and LC–MS, after a major section on sample preparation, and points out the growing use of high resolution MS especially in regard to LC–MS. The increasing use of high resolution MS is helping post-targeting and nontargeting analysis to emerge in the field.

Roger W. Giese
Boston, MA, USA
Available online 3 February 2017

Anexo II

Anexo II. Información suplementaria del articulo científico 2

Post-run target screening strategy for ultra high performance liquid chromatography coupled to Orbitrap based on food-packaging migrants analysis and quantitative analysis of primary aromatic amines.

Yovanna Sanchis[a,b], Clara Coscollà[a,b], Marta Roca [a,b] and Vicent Yusà[a,b,c*]

[a] Foundation for the Promotion of the Health and Biomedical Research in the Valencia Region (FISABIO-Public Health), 21, Avenida Catalunya, 46020 Valencia, Spain

[b]Public Health Laboratory of Valencia, 21, Avenida Catalunya, 46020 Valencia, Spain

c Analytical Chemistry Department, Universit of Valencia, Edifici Jeroni Muñoz,Dr. Moliner 50, 46100 Burjassot, Spain

Table SI-1. Exact mass database for post-run target screening including elemental composition, theoretical accurate mass and fragments of specific food-packaging migrants

Compound	CAS-number	Elemental composition	Monitored ion	Monitored mass	Fragment ion 1 (elemental composition)	ref	Fragment ion 2 (elemental composition)	ref
1,5-Diaminonaphthalene	2243-62-1	C10H10N2	M+H	159.09167	143.07287 (C10H9N)	[9]		
3,3' dimethylbenzidine	119-93-7	C14H16N2	M+H	213.1386	181.0802 (C12H9N2)	[9]	196.1102 (C13H12N2)	[5,9]
1-Hydroxycyclohexyl phenyl ketone	947-19-3	C13H16O2	M+H	205.1223	186.9958 (C12H11O2)	[23]		
2,2-dimethoxy-2-phenylacetophenone	24650-42-8	C16H16O3	M+H	257.11722	225.0910 (C15H13O2)			
2,4,5_trimethylaniline	137-17-7	C9H13N	M+H	136.11207	119.0855 (C9H11)	[5]		
2,4-Diethyl-9H-thioxanthen-9-one	82799-44-8	C17H16OS	M+H	269.09946	253.1045 (C17H17S)			
2,4-Toluenediamine	95-80-7	C7H10N2	M+H	123.09167	108.0682 (C6H8N2)	[5,9]	105.0573 (C7H7N)	
2,6-Dimethylaniline	87-62-7	C8H11N	M+H	122.09642	105.0698 (C8H9)	[5]	107.0855 (C8H11)	[5]
2,6-Toluenediamine	823-40-5	C7H10N2	M+H	123.09167	108.0682 (C6H8N2)	[5,9]	105.0573 (C7H7N)	
2-Ethylhexyl 4-(dimethylamino)benzoate	21245-02-3	C17H27NO2	M+H	278.21145	194.1175 (C11H16NO2)		263.1879 (C16H25NO2)	[25]
2H-perfluoro-2-decenoic acid	67-56-1	C10H2F16O2	M+H	458.9872	304.2182 (C7H30F10)	[24]		
2-Hydroxy-2-methylpropiophenone	7473-98-5	C10H12O2	M+H	164.08373	109.0647(C7H9O)			
2-Isopropyl Thioxanthone	5495-84-1	C16H14OS	M+H	255.08381	199.0575 (C13H11S)		213.0368 (C13H9O5)	[25]
2-Naphthylamine	91-59-8	C10H9N	M+H	144.08077	127.0542 (C10H7)			

Compound	CAS-number	Elemental composition	Monitored ion	Monitored mass	Fragment ion 1 (elemental composition)	ref	Fragment ion 2 (elemental composition)	ref
2-Perfluorooctyl ethanoic acid	Not available	C10H3F17O2	M+H	478.99345	389.0005 (C10H3F14)	[24]		
3,3-Dichlorobenzidine	91-94-1	C12H10Cl2N2	M+H	253.02938	236.0028 (C12H8Cl2N)		217.0527 (C12H10ClN2)	
3,3-Dimethoxybenzidine	119-90-4	C14H16N2O2	M+H	245.12118	198.0913 (C13H12NO)			
4,4'-Bis(dimethylamino)-benzophenone	90-94-8	C21H28N2O	M+H	325.22744	297.1961 (C19H25N2O)		309.1961 (C20H25N2O)	
4,4-Diaminodiphenyl ether	101-80-4	C12H12N2O	M+H	201.10223	108.0447 (C6H6ON)	[5,9]	184.0752 (C12H10ON)	[5]
4,4'-Methylene-bis(2-chloroaniline	101-14-4	C13H12Cl2N2	M+H	267.04503	126.0105 (C6H5ClN)		215.0496 (C13H10ClN)	
4,4-Methylene-bis-(2-Methylaniline)	838-88-0	C15H18N2	M+H	227.15427	120.0807 (C8H10N)	[5]		
4,4-Methylenedianiline	101-77-9	C13H14N2	M+H	199.12297	106.0652 (C7H8N)	[5,9]	76.0308 (C6H4)	[5]
4-(Methylthio)aniline	104-96-1	C7H9NS	M+H	140.04567	106.0651 (C7H8N)			
4-aminoazobenceno	60-09-3	C12H11N3	M+H	198.10257	105.0447 (C6H5N2)			
4-Aminobiphenyl	92-67-1	C12H11N	M+H	170.09642	153.0698 (C12H9)	[5]	152.06205 (C12H8)	[5]
4-BenzoyIbiphenyl	2128-93-0	C19H14O	M+H	259.11174	181.0647 (C13H9O)			
4-Chloro-o-toluidine	95-69-2	C7H8ClN	M+H	142.0418	125.0152 (C7H6Cl)			
4-Isopropylthioxanthone	83846-86-0	C16H14OS	M+H	255.08381	213.0368 (C13H9OS)	[25]		
4-Methoxy-m-phenylenediamine	615-05-4	C7H10N20	M+H	139.08658	107.0603 (C6H7N2)			
5-Nitro-o-Toluidine	99-55-8	C7H8N2O2	M+H	153.06585	106.0651 (C7H8N)			

Compound	CAS-number	Elemental composition	Monitored ion	Monitored mass	Fragment ion 1 (elemental composition)	ref	Fragment ion 2 (elemental composition)	ref
Benzidine	92-87-5	C12H12N2	M+H	185.10732	166.0651 (C12H8N)	[5]		
Benzophenone	119-61-9	C13H10O	M+H	183.08044	152.0620 (C12H8)			
Benzyl butyl phthalate	85-68-7	C19H20O4	M+H	313.14343	231.1016 (C14H15O3)		229.0859 (C14H13O3)	[25]
Bisphenol A	80-05-7	C15H16O2	M+H	228.11448	211.1117 (C15H15O)	[25]	107.0491 (C7H7O)	
Bisphenol A (2,3-dihydroxypropyl) glycidyl ether	76002-91-0	C21H26O5	M+H	358.17747	135.0804 (C9H11O)	[25]		
Bisphenol A (3-chloro-2-hydroxupropyl) (2,3-dihydrocypropyl) ether	227947-06-0	C21H27CLO5	M+H	394.15415	135.0804 (C9H11O)	[25]	107.0491 (C7H7O)	
Bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether	13836-48-1	C21H25CLO4	M+H	376.14358	227.0833 (C12H16ClO2)	[25]		
Bisphenol A bis (1,2-dihydroxypropyl) ether	5581-32-8	C21H28O6	M+H	376.18804	135.0804 (C9H11O)	[25]		
Bisphenol A bis(3-chloro-2-hydroxypropyl)ether	4809-35-2	C21H26CL2O4	M+H	412.12026	227.0833 (C12H16ClO2)	[25]		
Bisphenol A diclycidyl ether	1675-54-3	C21H24O4	M+H	340.16691	107.0491(C7H7O)			
Bisphenol B	77-40-7	C16H18O2	M+H	242.13013	149.0961 (C10H13O)			
Bisphenol E	2081-08-5	C14H14O2	M+H	214.09883	121.0648 (C8H9O)			
Bisphenol F	620-92-8	C13H14O2	M+H	202.09883	168.0569 (C12H8O)	[25]	107.0491(C7H7O)	
Bisphenol F bis(2,3-dihydroxypropyl) ether	72406-26-9	C21H28O6	M+H	376.18804	201.0910 (C13H13O2)			
Bisphenol F bis(3-chloro-2-hydroxypropyl)ether	Not available	C21H26CL2O4	M+H	412.12026	202.09883 (C13H14O2)			

Compound	CAS-number	Elemental composition	Monitored ion	Monitored mass	Fragment ion 1 (elemental composition)	ref	Fragment ion 2 (elemental composition)	ref
Bisphenol F diglycidyl ether	2095-03-6	C19H20O4	M+H	312.13561	201.0910 (C13H13O2)	[25]		
Bisphenol S	80-09-1	C12H10O4S	M+H	250.02943	156.9954 (C6H5O3S)			
di-2-ethylhexyl phthalate	117-81-7	C24H38O4	M+H	391.28428	279.1597 (C16H23O4)		149.0237 (C8H5O3)	
di-isodecyl phthalate	26761-40-0	C28H46O4	M+H	447.34688	289.1798 (C18H25O3)		261.1798 (C17H25O2)	
di-isononyl phthalate	68515-48-0	C26H42O4	M+H	419.31558	275.1598 (C17H23O3)			
di-n-butyl phthalate	84-74-2	C16H22O4	M+H	279.15908	149.0233 (C8H5O3)			
Ethyl 4-dimethylaminobenzoate	10287-53-3	C11H15NO2	M+H	194.11755	120.0808 (C8H10N)			
m-Phenylenediamine	108-45-2	C6H8N2	M+H	109.07602	92.0495 (C6H6N)	[5]		
o-Aminoazotoluene	97-56-3	C14H15N3	M+H	226.13387	106.0651 (C7H8N)			
2-Anisidine	104-94-9	C7H9NO	M+H	124.07569	107.0491 (C7H7O)			
o-Dianisidine	119-90-4	C14H16N2O2	M+H	245.12845	121.0522 (C7H7NO)	[7]		
o-Toludine	95-53-4	C7H9N	M+H	108.08077	91.0542 (C7H7)	[5,7]		
p-Aminoazobenzene	60-09-3	C12H11N3	M+H	198.10257	183.0889 (C12H11N2)			
p-Chloroaniline	106-47-8	C6H6ClN	M+H	128.02615	110.9996 (C6H4Cl)			
Perfluoro-1-butanesulfonate	29420-49-3	C4HF9SO3	M+H	300.95754	79.0917 (C4H11F)	[24]		
Perfluorobutanoic acid	375-22-4	C4HF7O2	M+H	214.99375	198.9980 (C4HF7O)	[24]		
Perfluorodecane sulfonate	1763-23-1	C10H2F20	M+H	502.99099	91.0917 (C5H11F)	[24]		

Compound	CAS-number	Elemental composition	Monitored ion	Monitored mass	Fragment ion 1 (elemental composition)	ref	Fragment ion 2 (elemental composition)	ref
Perfluorodecanoic acid	2058-94-8	C10HF19O2	M+H	514.97459	467.9812 (C9HF18O)	[24]		
Perfluorododecanoic acid	307-55-1	C12HF23O2	M+H	614.9682	545.9729 (C11HF20O2)	[24]		
Perfluoroheptanoic acid	375-85-9	C7HF13O2	M+H	364.98417	317.9905 (C6HF12O)	[24]		
Perfluorohexane sulfonate	355-46-4	C6F13O3S	M+H	339.94333	83.0666 (C3F2H8)	[24]		
Perfluorohexanoic acid	307-24-4	C6HF11O2	M+H	314.98737	298.9924 (C6HF11O)	[24]		
Perfluorononanoic acid	375-95-1	C9HF17O2	M+H	464.97778	419.9801 (C8F17)	[24]		
Perfluorooctane sulfonamide	754-91-6	C8H2F17NO2S	M+H	499.96075	79.0917 (C4H11F)	[24]		
Perfluorooctane sulfonate	1763-23-1	C8HF17O3S	M+H	500.94477	95.9839 (C2HF2S)	[24]		
Perfluorooctanoic acid	335-67-1	C8HF15O2	M+H	414.98097	367.9879 (C7HF14O)	[24]		
Perfluoropentanoic acid	2706-90-3	C5HF9O2	M+H	264.99055	248.9956 (C5HF9O)	[24]		
Perfluorotridecanoic acid	72629-94-8	C13HF25O2	M+H	664.96501	620.9751 (C12HF25)	[24]		
Perfluoroundecanoic acid	2058-94-8	C11HF21O2	M+H	564.97139	520.9815 (C10HF21)	[24]		
Perfluortetradecanoic acid	376-06-7	C14HF27O2	M+H	714.95181	668.0248 (C14H8F25O)	[24]		

Anexo III

Anexo III. Información suplementaria del articulo científico 3

Comprehensive analysis of Photoinitiators and PAAS in contact materials using Liquid Chromatography High-Resolution Mass Spectrometry

Yovana Sanchis[a], Clara Coscollà[a], Vicent Yusà[a,b]

[a] *Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020 Valencia, Spain*

[b] *Analytical Chemistry Department, Universit of Valencia, Edifici Jeroni Muñoz,Dr. Moliner 50, 46100 Burjassot, Spain*

Table SI-1. Exact mass database for post-run target screening including elemental composition, theoretical accurate mass and fragments of specific food-packaging migrants

Compound	Elemental composition	Monitored ion	Monitored mass	Fragment ion 1 (elemental composition)	ref	Fragment ion 2 (elemental composition)	ref
1,5-Diaminonaphthalene	C10H10N2	$[M+H]^+$	159.09167	143.07287 (C10H9N)	[3]		
3,3' dimethylbenzidine	C14H16N2	$[M+H]^+$	213.1386	181.0802 (C12H9N2)	[3]	196.1102 (C13H12N2)	[3,4]
1-Hydroxycyclohexyl phenyl ketone	C13H16O2	$[M+H]^+$	205.1223	186.9958 (C12H11O2)	[9]		
2,2-dimethoxy-2-phenylacetophenone	C16H16O3	$[M+H]^+$	257.11722	225.0910 (C15H13O2)			
2,4,5_trimethylaniline	C9H13N	$[M+H]^+$	136.11207	119.0855 (C9H11)	[3]		
2,4-Diethyl-9H-thioxanthen-9-one	C17H16OS	$[M+H]^+$	269.09946	253.1045 (C17H17S)			
2,4-Toluenediamine	C7H10N2	$[M+H]^+$	123.09167	108.0682 (C6H8N2)	[3,4]	105.0573 (C7H7N)	
2,6-Dimethylaniline	C8H11N	$[M+H]^+$	122.09642	105.0698 (C8H9)	[3]	107.0855 (C8H11)	[4]
2,6-Toluenediamine	C7H10N2	$[M+H]^+$	123.09167	108.0682 (C6H8N2)	[3,4]	105.0573 (C7H7N)	
2-Ethylhexyl 4-(dimethylamino)benzoate	C17H27NO2	$[M+H]^+$	278.21145	194.1175 (C11H16NO2)		263.1879 (C16H25NO2)	[9]
2H-perfluoro-2-decenoic acid	C10H2F16O2	$[M+H]^+$	458.9872	304.2182 (C7H30F10)	[9]		
2-Hydroxy-2-methylpropiophenone	C10H12O2	$[M+H]^+$	164.08373	109.0647(C7H9O)			
2-Isopropyl Thioxanthone	C16H14OS	$[M+H]^+$	255.08381	199.0575 (C13H11S)		213.0368 (C13H9O5)	[9]
2-Methoxy-5-methylaniline	C8H11NO	$[M+H]^+$	138.09134	121.0647 (C8H9O)	[3]	106.04132 (C7H6O)	[3]
2-Naphthylamine	C10H9N	$[M+H]^+$	144.08077	127.0542 (C10H7)			

2-Perfluorooctyl ethanoic acid	C10H3F17O2	$[M+H]^+$	478.99345	389.0005 (C10H3F14)	[9]		
3,3-Dichlorobenzidine	C12H10Cl2N2	$[M+H]^+$	253.02938	236.0028 (C12H8Cl2N)		217.0527 (C12H10ClN2)	
3,3-Dimethoxybenzidine	C14H16N2O2	$[M+H]^+$	245.12118	198.0913 (C13H12NO)			
4,4'-Bis(dimethylamino)-benzophenone	C21H28N2O	$[M+H]^+$	325.22744	297.1961 (C19H25N2O)		309.1961 (C20H25N2O)	
4,4-Diaminodiphenyl ether	C12H12N2O	$[M+H]^+$	201.10223	108.0447 (C6H6ON)	[3,4]	184.0752 (C12H10ON)	[3]
4,4'-Methylene-bis(2-chloroaniline	C13H12Cl2N2	$[M+H]^+$	267.04503	126.0105 (C6H5ClN)		215.0496 (C13H10ClN)	
4,4-Methylene-bis-(2-Methylaniline)	C15H18N2	$[M+H]^+$	227.15427	120.0807 (C8H10N)	[3]		
4,4-Methylenedianiline	C13H14N2	$[M+H]^+$	199.12297	106.0652 (C7H8N)	[3,4]	76.0308 (C6H4)	[3]
4-(Methylthio)aniline	C7H9NS	$[M+H]^+$	140.04567	106.0651 (C7H8N)			
4-aminoazobenceno	C12H11N3	$[M+H]^+$	198.10257	105.0447 (C6H5N2)			
4-Aminobiphenyl	C12H11N	$[M+H]^+$	170.09642	153.0698 (C12H9)	[3]	152.06205 (C12H8)	[3]
4-BenzoylbiphenyI	C19H14O	$[M+H]^+$	259.11174	181.0647 (C13H9O)			
4-Chloro-o-toluidine	C7H8ClN	$[M+H]^+$	142.0418	125.0152 (C7H6Cl)			
4-Isopropylthioxanthone	C16H14OS	$[M+H]^+$	255.08381	213.0368 (C13H9OS)	[9]		
4-Methoxy-m-phenylenediamine	C7H10N20	$[M+H]^+$	139.08658	107.0603 (C6H7N2)			
5-Nitro-o-Toluidine	C7H8N2O2	$[M+H]^+$	153.06585	106.0651 (C7H8N)			
Aniline	C6H7N	$[M+H]^+$	94.05125	76.0308 (C6H4)	[9]		
Benzidine	C12H12N2	$[M+H]^+$	185.10732	166.0651 (C12H8N)	[3]		
Benzophenone	C13H10O	$[M+H]^+$	183.08044	152.0620 (C12H8)			
Benzyl butyl phthalate	C19H20O4	$[M+H]^+$	313.14343	231.1016 (C14H15O3)		229.0859 (C14H13O3)	[9]

Bisphenol A	C15H16O2	$[M-H]^-$	228.11448	211.1117 (C15H15O)	[9]	107.0491 (C7H7O)
Bisphenol A (2,3-dihydroxypropyl) glycidyl ether	C21H26O5	$[M-H]^-$	358.17747	135.0804 (C9H11O)	[9]	
Bisphenol A (3-chloro-2-hydroxupropyl) (2,3-dihydrocypropyl) ether	C21H27CLO5	$[M-H]^-$	394.15415	135.0804 (C9H11O)	[9]	107.0491 (C7H7O)
Bisphenol A (3-chloro-2-hydroxypropyl) glycidyl ether	C21H25CLO4	$[M-H]^-$	376.14358	227.0833 (C12H16ClO2)	[9]	
Bisphenol A bis (1,2-dihydroxypropyl) ether	C21H28O6	$[M-H]^-$	376.18804	135.0804 (C9H11O)	[9]	
Bisphenol A bis(3-chloro-2-hydroxypropyl)ether	C21H26CL2O4	$[M-H]^-$	412.12026	227.0833 (C12H16ClO2)	[9]	
Bisphenol A diclycidyl ether	C21H24O4	$[M-H]^-$	340.16691	107.0491(C7H7O)		
Bisphenol B	C16H18O2	$[M-H]^-$	242.13013	149.0961 (C10H13O)		
Bisphenol E	C14H14O2	$[M-H]^-$	214.09883	121.0648 (C8H9O)		
Bisphenol F	C13H14O2	$[M-H]^-$	202.09883	168.0569 (C12H8O)	[9]	107.0491(C7H7O)
Bisphenol F bis(2,3-dihydroxypropyl) ether	C21H28O6	$[M-H]^-$	376.18804	201.0910 (C13H13O2)		
Bisphenol F bis(3-chloro-2-hydroxypropyl)ether	C21H26CL2O4	$[M-H]^-$	412.12026	202.09883 (C13H14O2)		
Bisphenol F diglycidyl ether	C19H20O4	$[M-H]^-$	312.13561	201.0910 (C13H13O2)	[9]	
Bisphenol S	C12H10O4S	$[M-H]^-$	250.02943	156.9954 (C6H5O3S)		
di-2-ethylhexyl phthalate	C24H38O4	$[M+H]^+$	391.28428	279.1597 (C16H23O4)		149.0237 (C8H5O3)
di-isodecyl phthalate	C28H46O4	$[M+H]^+$	447.34688	289.1798 (C18H25O3)		261.1798 (C17H25O2)

di-isononyl phthalate	C26H42O4	$[M+H]^+$	419.31558	275.1598 (C17H23O3)	
di-n-butyl phthalate	C16H22O4	$[M+H]^+$	279.15908	149.0233 (C8H5O3)	
Ethyl 4-dimethylaminobenzoate	C11H15NO2	$[M+H]^+$	194.11755	120.0808 (C8H10N)	
m-Phenylenediamine	C6H8N2	$[M+H]^+$	109.07602	92.0495 (C6H6N)	[3]
o-Aminoazotoluene	C14H15N3	$[M+H]^+$	226.13387	106.0651 (C7H8N)	
2-Anisidine	C7H9NO	$[M+H]^+$	124.07569	107.0491 (C7H7O)	
o-Dianisidine	C14H16N2O2	$[M+H]^+$	245.12845	121.0522 (C7H7NO)	[6]
o-Toludine	C7H9N	$[M+H]^+$	108.08077	91.0542 (C7H7)	[5,6]
p-Aminoazobenzene	C12H11N3	$[M+H]^+$	198.10257	183.0889 (C12H11N2)	
p-Chloroaniline	C6H6ClN	$[M+H]^+$	128.02615	110.9996 (C6H4Cl)	
Perfluoro-1-butanesulfonate	C4HF9SO3	$[M+H]^+$	300.95754	79.0917 (C4H11F)	[9]
Perfluorobutanoic acid	C4HF7O2	[M-H]-	214.99375	198.9980 (C4HF7O)	[9]
Perfluorodecane sulfonate	C10H2F20	[M-H]-	502.99099	91.0917 (C5H11F)	[9]
Perfluorodecanoic acid	C10HF19O2	[M-H]-	514.97459	467.9812 (C9HF18O)	[9]
Perfluorododecanoic acid	C12HF23O2	[M-H]-	614.9682	545.9729 (C11HF20O2)	[9]
Perfluoroheptanoic acid	C7HF13O2	[M-H]-	364.98417	317.9905 (C6HF12O)	[9]
Perfluorohexane sulfonate	C6F13O3S	[M-H]-	339.94333	83.0666 (C3F2H8)	[9]
Perfluorohexanoic acid	C6HF11O2	[M-H]-	314.98737	298.9924 (C6HF11O)	[9]
Perfluorononanoic acid	C9HF17O2	[M-H]-	464.97778	419.9801 (C8F17)	[9]
Perfluorooctane sulfonamide	C8H2F17NO2S	[M-H]-	499.96075	79.0917 (C4H11F)	[9]

Perfluorooctane sulfonate	C8HF17O3S	[M-H]-	500.94477	98.9551 (FO3S-)	[9]
Perfluorooctanoic acid	C8HF15O2	[M-H]-	412.96643	368.97660(C7HF14O)	[9]
Perfluoropentanoic acid	C5HF9O2	[M-H]-	264.99055	248.9956 (C5HF9O)	[9]
Perfluorotridecanoic acid	C13HF25O2	[M-H]-	664.96501	620.9751 (C12HF25)	[9]
Perfluoroundecanoic acid	C11HF21O2	[M-H]-	564.97139	520.9815 (C10HF21)	[9]
Perfluortetradecanoic acid	C14HF27O2	[M-H]-	714.95181	668.0248 (C14H8F25O)	[9]
trimethyl phosphate	C3H9O4P	[M+H]+	141,03112	127,01547 (C2H8O4P+)	
triethyl phosphate	C6H15O4P	[M+H]+	183,07807	98,98467 (H4O4P+)	
tris(2-chloroethyl) phosphate	C6H12Cl3O4P	[M+H]+	284,96116	98,98467 (H4O4P+)	
tri-isopropyl phosphate	C9H21O4P	[M+H]+	225,12502	98,98467 (H4O4P+)	
tri-n-propyl phosphate	C9H21O4P	[M+H]+	225,12502	98,98467 (H4O4P+)	
tris(2-choloroisopropyl) phosphate	C9H18Cl3O4P	[M+H]+	327,00811	98,98467 (H4O4P+)	
triphenyl phosphate	C18H15O4P	[M+H]+	327,07807	153,06988 (C12H9+)	
tri-n-butyl phosphate	C12H27O4P	[M+H]+	267,17197	98,98467 (H4O4P+)	
tris(2-butoxyethyl) phosphate	C18H39O4P	[M+H]+	399,25062	199,07299 (C6H16O5P+)	
2-ethylhexyl diphenyl phosphate	C20H27O4P	[M+H]+	363.1720	251.0468 (C12H11O4P)	

Anexo IV

Anexo IV. Información suplementaria del articulo científico 4

Analysis of four Parabens and Bisphenols A, F, S in urine, using Dilute and Shoot and liquid chromatography coupled to mass spectrometry.

Yovana Sanchis[a] , Clara Coscollà[a] ,Vicent Yusà[a,b]

[a] *Public Health Laboratory of Valencia-FISABIO, 21, Avenida Catalunya, 46020 Valencia, Spain*

[b] *Analytical Chemistry Department, Universit of Valencia, Edifici Jeroni Muñoz,Dr. Moliner 50, 46100 Burjassot, Spain*

Supplementary data :

- Table_SI-1. Optimization of APCI ionization source settings: Central Composite Design
- Table_SI-2. Matrix effect
- Figure_SI-1 : Matrix effect for BPS
- Figure_SI-2 : Matrix effect for BPA
- Figure_SI-3 : Matrix effect for BPF
- Figure_SI-4 : Matrix effect for BP
- Figure_SI-5 : Matrix effect for EP
- Figure_SI-6 : Matrix effect for MP
- Figure_SI-7 : Matrix effect for PP

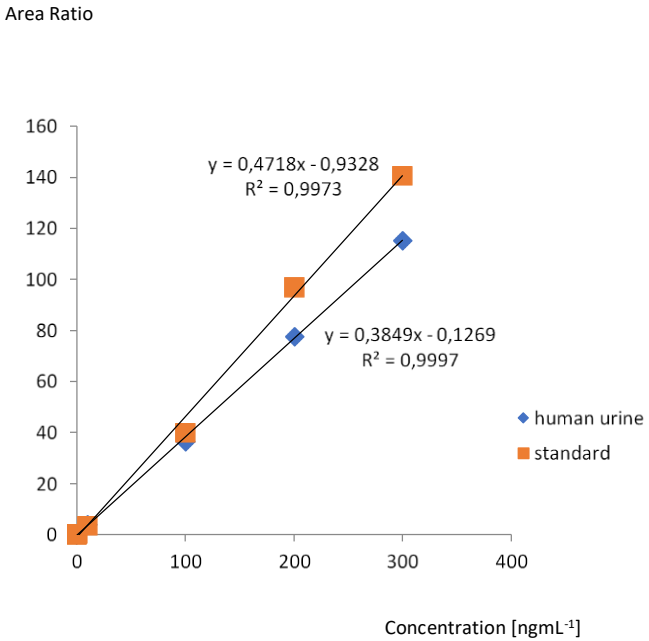
Table_SI-1. Optimization of APCI ionization source settings: Central Composite Design

Run	Sheath gas pressure (psi)	Discharge current(kV)	Auxiliary gas flow(a. u.)	Capillary temperature (ºC)	Vaporizat iontempe rature (ºc)	BPA(m/z133)	BPS (m/z 92.1)	BPF (m/z 105.1)	MP (m/z 136)	EP(m/z 136)	PP (m/z 136)	BP (m/z 136)
1	60	6	1	300	287	175244	562451	75388	1150250	221050	1199608	1112402
2	25	4	3	150	500	175052	577675	75379	1419900	12286	1533032	1534256
3	60	4	3	300	375	0	35239	8959	154441	0	295207	272637
4	45	4	4	225	287	145951	616004	68494	1602805	5458	1872889	1704882
5	45	4	0	225	375	232858	838918	101002	2149896	22896	1826532	1675851
6	60	4	3	150	375	156629	601711	64795	1642030	11833	1885581	1744434
7	60	4	3	150	500	140965	73982	66462	2258572	11014	3676737	3945988
8	60	4	4	300	287	139492	297000	50135	621127	0	687893	740235
9	25	4	1	300	463	4035	7328	10029	126905	10944	344931	404943
10	25	1	3	150	375	92972	179400	43209	494666	0	225903	169121
11	25	2	3	150	375	27736	34592	31069	293457	0	424047	437049
12	45	2	0	225	375	24364	41013	24213	348760	0	532307	479794
13	25	4	3	300	375	160816	630364	64817	1917598	181164	2043022	1933344
14	60	5	1	150	287	174456	469310	60926	884966	150361	744540	691714
15	25	4	4	300	463	169219	704659	68310	1939105	14972	1837597	1518458
16	60	5	5	300	375	110897	639653	54674	2268795	35365	2636373	1450628
17	25	5	1	150	463	20208	49798	25373	389627	0	813615	824552
18	60	2	5	300	375	123741	785580	60990	2961491	12110	2340317	863572
19	45	4	3	225	375	181066	781895	79243	2010017	19449	1915652	1866710
20	45	5	3	225	375	174724	732236	82324	2243006	125893	1959065	1530885
21	60	2	3	150	375	192742	477141	76445	955343	2213	474777	411619
22	60	2	3	150	375	159844	431398	57229	11480766	588	520127	556393
23	60	5	3	300	250	39785	79412	43339	394592	21319	860004	952253
24	25	5	3	300	250	24928	38607	23739	244447	2444	514785	601826
25	25	2	3	150	375	244094	1130428	112012	3664747	16320	2050746	1457251
26	25	5	4	150	463	164738	561013	65663	1156150	7126	1264879	1042417
27	45	5	1	225	287	23549	68639	24894	401562	22152	1151287	982853
28	25	2	3	300	500	57628	84163	52175	568516	31196	734918	755387

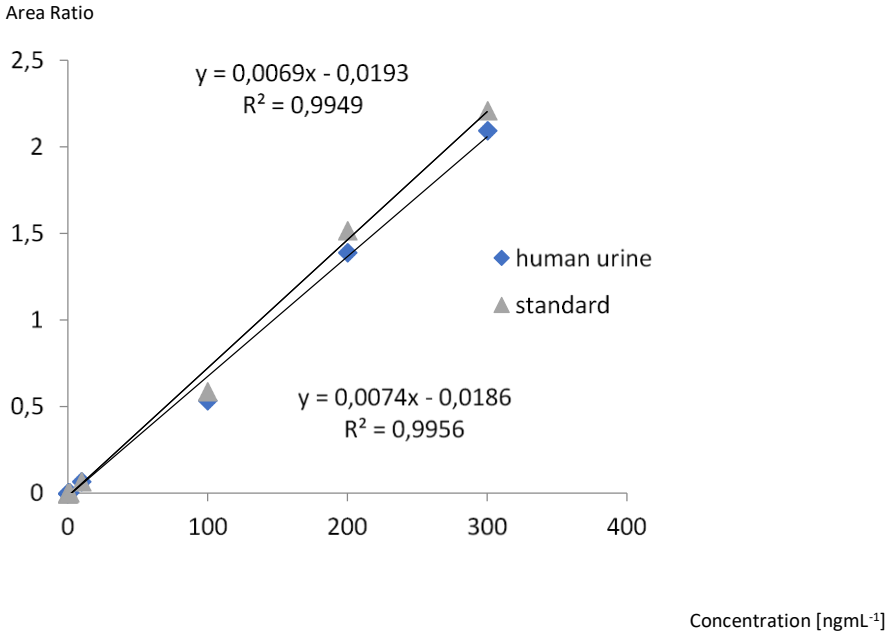
Table_SI-2. Matrix effect

Compounds	Slope	Matrix effect (%ME)
BPS	Urine : 0.3849 Std. in Water[a] : 0.4718	-19
BPA	Urine : 0.0074 Std in Water[a] : 0.0069	-7
BPF	Urine : 0.4756 Std. in Water[a] : 0.579	-18
BP	Urine : 0.0526 Std. in Water[a] : 0.0618	-15
EP	Urine : 0.7587 Std. in Water[a] : 0.9293	-18
MP	Urine : 0.062 Std. in Water[a] : 0.0668	-7
PP	Urine : 0.0672 Std. in Water[a] : 0.0629	-6

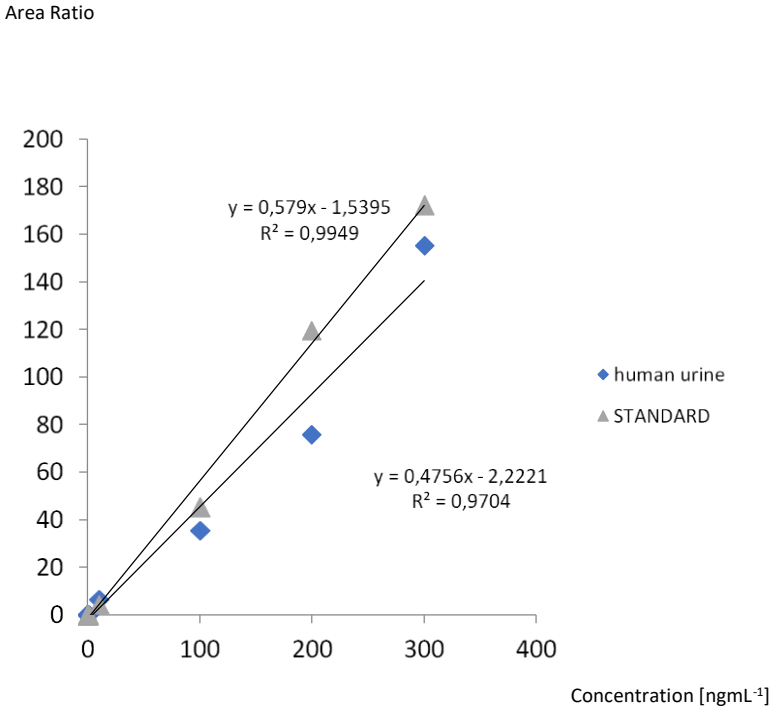
a : Calibration standards in water



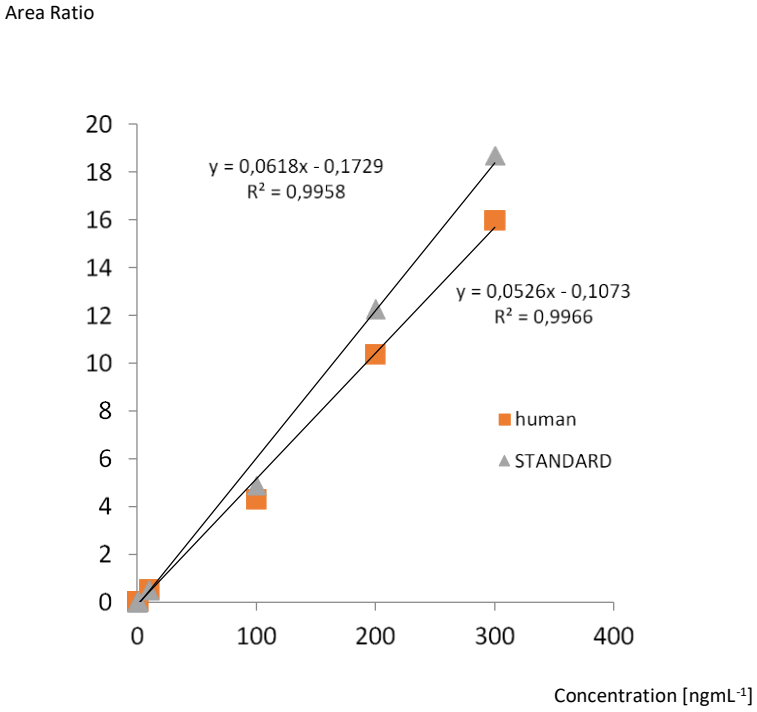
Figure_SI-1 : Matrix effect for BPS



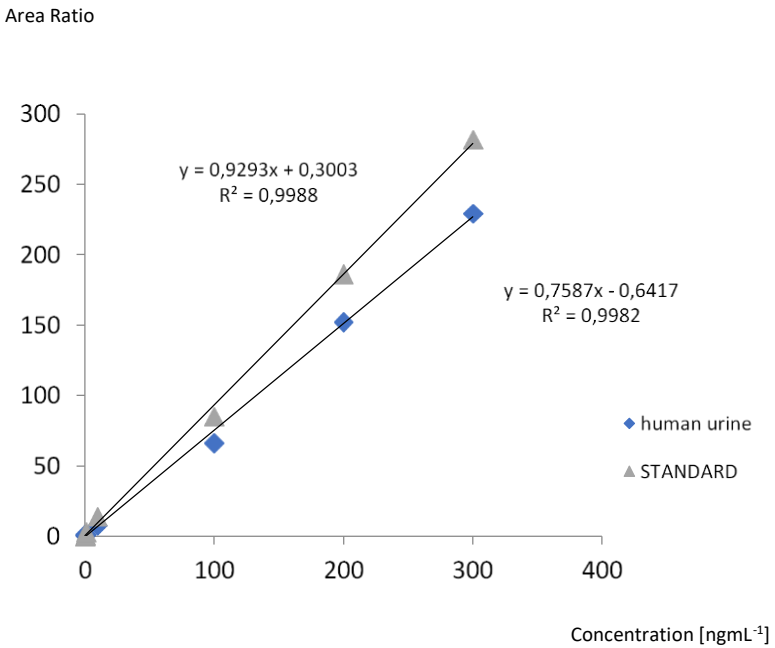
Figure_SI-2 : Matrix effect for BPA



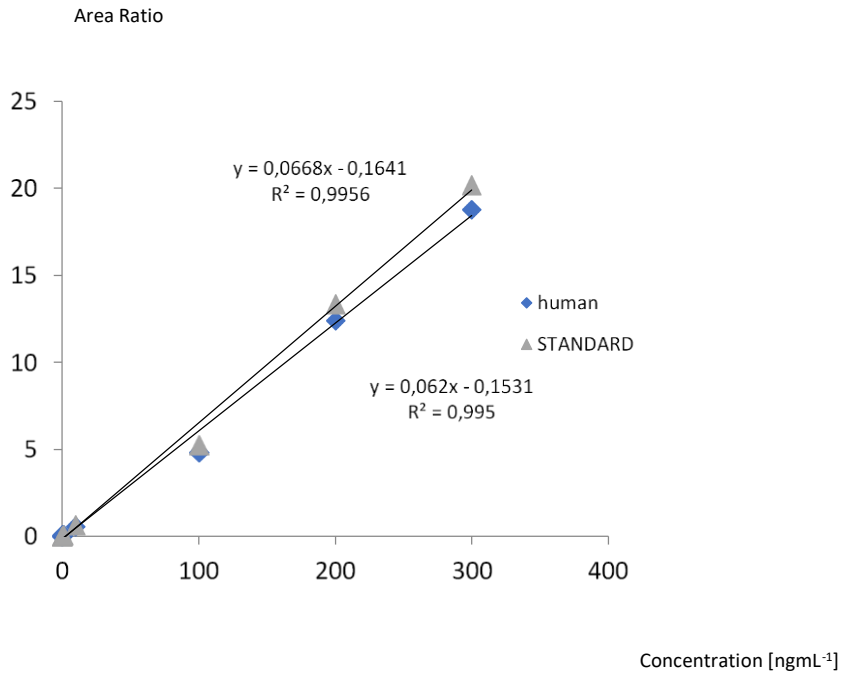
Figure_SI-3 : Matrix effect for BPF



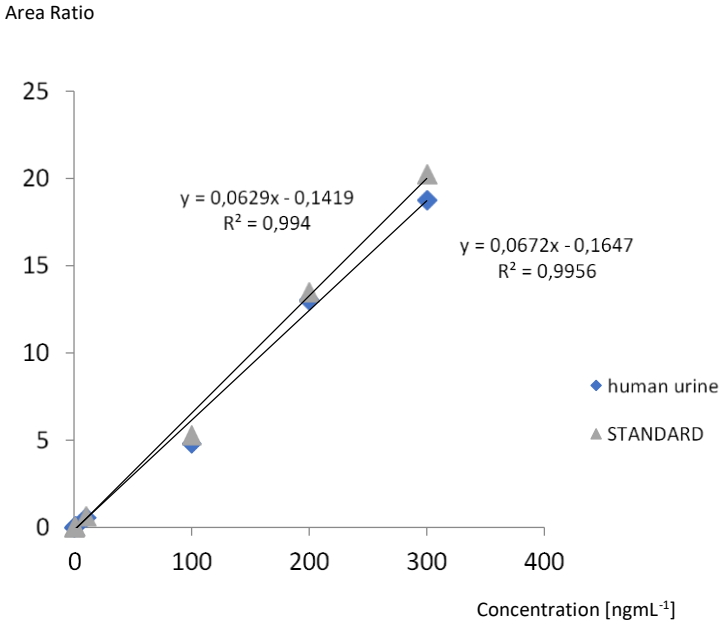
Figure_SI-4 : Matrix effect for BP



Figure_SI-5 : Matrix effect for EP



Figure_SI-6 : Matrix effect for MP



Figure_SI-7 : Matrix effect for PP

Anexo V

Anexo V. Información suplementaria del artículo cientíco 5

1. Características de la población (Table SI-1, Table SI-2)
2. Diagnosis y validación de los modelos de regresión lineal múltiple para bisfenoles y parabenos
3. Modelos de regresión para el bisfenol A e interacción con los factores de influencia.
4. Modelos de regresión para metilparabeno, etilparabeno y propilparabeno e interacción con los factores de influencia.

1) Características de la población

Table SI-1. Food consumption by groups in the population (grams/month).

Food consumption by groups (g/month) *Missing data, n(%): 6 (5.82 %)*	Median (minimum - maximum)
eggs	720 (30 - 1800)
Dairy products	13730 (1403.5 - 43446)
Meat products	5492.5 (737.5 - 13710)
Fishing products	4250 (1040 - 15640)
Vegetables	13146.5 (2982 - 38906)
Fruits	17062 (3948 - 84484)
Legumes and cereals	5017.5 (2047.5 - 26960)
Oils and fats	782.5 (152.5 - 1541.25)
Pastries	1217.5 (284 - 5645)
Miscellany	1360.5 (403 - 14619)
Drinks	44320 (4027.5 - 76562.5)
Packaged products (72h)	**13.5 (1 - 39)**

Table SI-2. Frequency of use of cosmetics products.

Cosmetics products	**n (%)**
Skin care	
Frecuency	
Never or before pregnancy	22 (21.36 %)
Daily	58 (56.31 %)
Several times a week	18 (17.47 %)
Sometimes in the month	4 (3.88 %)
Missing data	1 (0.97 %)
Parfums	
Frecuency	
Never or before pregnancy	48 (46.60 %)
Daily	28 (27.18 %)
Several times a week	20 (19.41 %)
Sometimes in the month	4 (3.88 %)
Missing data	3 (2.91 %)
Deodorants	
Frecuency	
Never or before pregnancy	6 (5.82 %)
Daily	83 (80.58 %)
Several times a week	5 (4.85 %)
Missing data	9 (8.74 %)
Sun screen	
Frecuency	
Never or before pregnancy	62 (60.19 %)
Daily	15 (14.56 %)
Several times a week	10 (9.71 %)
Sometimes in the month	2 (1.94 %)
Ocasionally	9 (8.74 %)

Cosmetics products	n (%)
Missing data	5 (4.85 %)
Hear colour	
Times/year	1 (0 - 26)[a]
Missing data	12 (11.65 %)
Last application	
≤1 week	9 (8.74 %)
< 1 month	7 (6.80 %)
≥ 1 month, < 3 months	27 (26.21 %)
≥ 3 months	12 (11.65 %)
Never or before pregnancy	45 (43.69 %)
Missing data	3 (2.91 %)
Lipstick	
Frecuency	
Never or before pregnancy	75 (72.82 %)
Daily	10 (9.71 %)
Several times a week	7 (6.8 %)
Sometimes in the month	11 (10.68 %)
Makeup	
Frecuency	
Never or before pregnancy	57 (55.34 %)
Daily	13 (12.62 %)
Several times a week	14 (13.59 %)
Sometimes in the month	17 (16.50 %)
Missing data	2 (1.94 %)

[a]Values expressed as median (minimum - maximum).

2) Diagnosis y validación de los modelos de regresión lineal múltiple

Para que los resultados de los modelos de regresión puedan considerarse válidos, los residuos (diferencia entre los valores observados de los niveles de contaminantes y los estimados por los modelos) deben verificar las siguientes hipótesis: normalidad, varianza constante, media 0 e independencia.

A continuación, verificamos las hipótesis anteriores en cada uno de los modelos considerados.

Log(methylparaben)

- **Normalidad**

Para contrastar si los residuos del modelo se ajustan a una distribución Normal utilizamos el test de Shapiro-Wilk.

Shapiro-Wilk normality test:
data: fit_final$residuals
W = 0.96972, p-value = 0.1875
El p-valor del test es 0.1875 > 0.05, por lo que, no puede rechazarse la normalidad de los residuos.

- **Varianza constante**

Utilizamos el test de Breusch-Pagan para contrastar si los residuos tienen varianza constante.

studentized Breusch-Pagan test
data: fit_final
BP = 14.494, df = 16, p-value = 0.5619
El p-valor del test es 0.5619 > 0.05, por tanto, no puede rechazarse que los residuos tengan varianza constante.

- **Media 0**

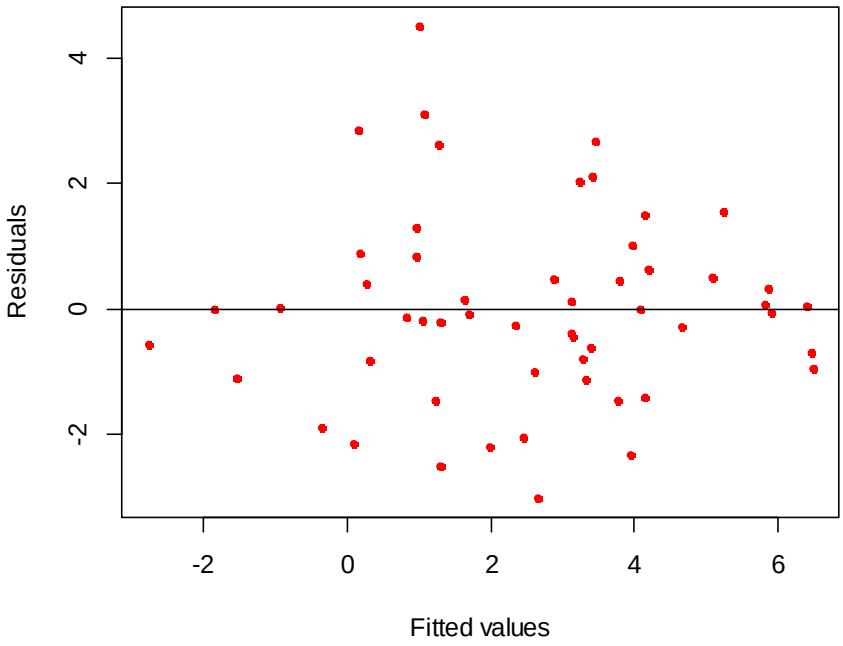
Para contrastar si los residuos tienen media 0, utilizamos el test t-Student.

One Sample t-test
data: fit_final$residuals
t = 1.2268e-16, df = 53, p-value = 1
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 -0.4154845 0.4154845
sample estimates:
mean of x
2.541275e-17

El p-valor del test es 1 > 0.05 por lo que no puede rechazarse que los residuos tengan media 0.

- **Independencia**

Para comprobar que los residuos son independientes los representamos gráficamente frente a los valores ajustados.



No se observa ninguna tendencia en este gráfico, por tanto, se verifica la independencia de los residuos.

Log(ethylparaben)

- **Normalidad**

Para contrastar si los residuos del modelo se ajustan a una distribución Normal utilizamos el test de Shapiro-Wilk.

Shapiro-Wilk normality test

data: fit_final$residuals

W = 0.98724, p-value = 0.4919

El p-valor del test es 0.4919 > 0.05, por lo que, no puede rechazarse la normalidad de los residuos.

- **Varianza constante**

Utilizamos el test de Breusch-Pagan para contrastar si los residuos tienen varianza constante.

studentized Breusch-Pagan test
data: fit_final
BP = 1.7916, df = 3, p-value = 0.6168
El p-valor del test es 0.6168 > 0.05, por tanto, no puede rechazarse que los residuos tengan varianza constante.

- **Media 0**

Para contrastar si los residuos tienen media 0, utilizamos el test t-Student.

One Sample t-test

data: fit_final$residuals

t = 3.8818e-16, df = 94, p-value = 1

alternative hypothesis: true mean is not equal to 0

95 percent confidence interval:

-0.392045 0.392045

sample estimates:

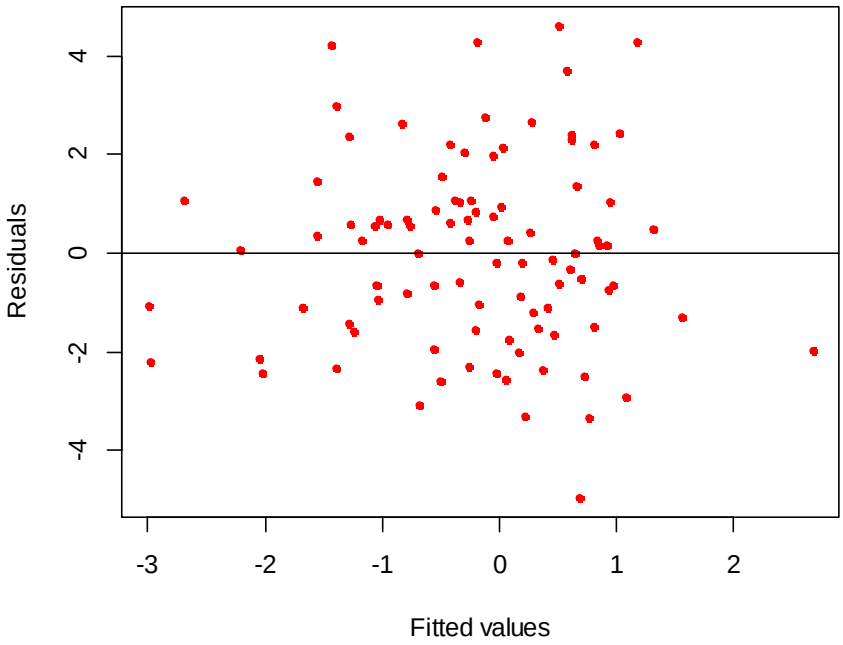
mean of x

7.664739e-17

El p-valor del test es 1 > 0.05 por lo que no puede rechazarse que los residuos tengan media 0.

- **Independencia**

Para comprobar que los residuos son independientes los representamos gráficamente frente a los valores ajustados.



No se observa ninguna tendencia en este gráfico, por tanto, se verifica la independencia de los residuos.

Log(propylparaben)

- **Normalidad**

Para contrastar si los residuos del modelo se ajustan a una distribución Normal utilizamos el test de Shapiro-Wilk.

Shapiro-Wilk normality test
data: fit_final$residuals
W = 0.96947, p-value = 0.06068
El p-valor del test es 0.06 > 0.05, por lo que, no puede rechazarse la normalidad de los residuos.

- **Varianza constante**

Utilizamos el test de Breusch-Pagan para contrastar si los residuos tienen varianza constante.

studentized Breusch-Pagan test
data: fit_final
BP = 1.1723, df = 2, p-value = 0.5565

El p-valor del test es 0.5565 > 0.05, por tanto, no puede rechazarse que los residuos tengan varianza constante.

- **Media 0**

Para contrastar si los residuos tienen media 0, utilizamos el test t-Student.

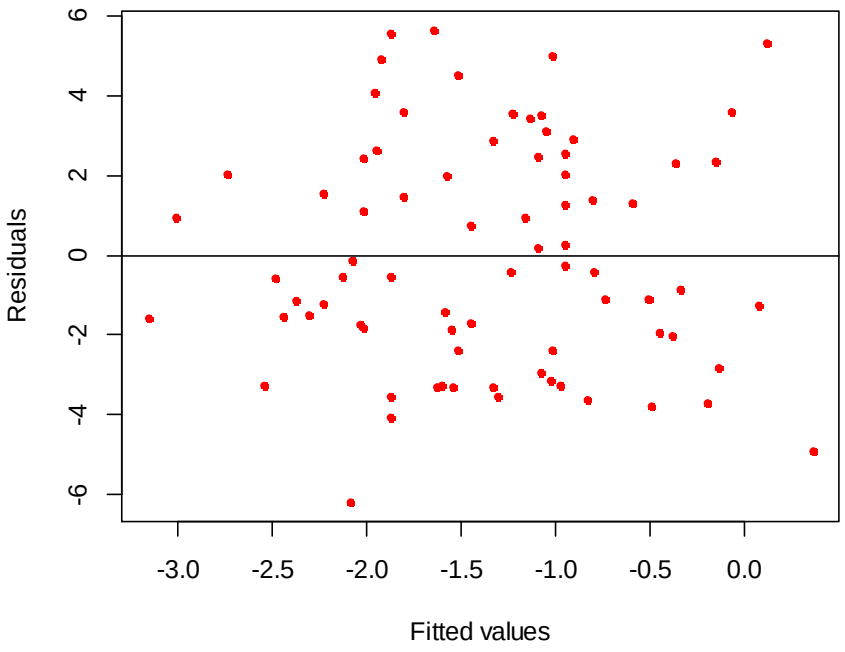
```
        One Sample t-test

data: fit_final$residuals
t = -1.5659e-16, df = 76, p-value = 1
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 -0.6455695 0.6455695
sample estimates:
 mean of x
-5.075756e-17
```

El p-valor del test es 1 > 0.05 por lo que no puede rechazarse que los residuos tengan media 0.

- **Independencia**

Para comprobar que los residuos son independientes los representamos gráficamente frente a los valores ajustados.



No se observa ninguna tendencia en este gráfico, por tanto, se verifica la independencia de los residuos.

Log(bisphenol A)

- **Normalidad**

Para contrastar si los residuos del modelo se ajustan a una distribución Normal utilizamos el test de Shapiro-Wilk.

Shapiro-Wilk normality test
data: fit_final$residuals
W = 0.98124, p-value = 0.2022
El p-valor del test es 0.2022 > 0.05, por lo que, no puede rechazarse la normalidad de los residuos.

- **Varianza constante**

Utilizamos el test de Breusch-Pagan para contrastar si los residuos tienen varianza constante.

studentized Breusch-Pagan test
data: fit_final
BP = 3.6348, df = 5, p-value = 0.6031
El p-valor del test es 0.6031 > 0.05, por tanto, no puede rechazarse que los residuos tengan varianza constante.

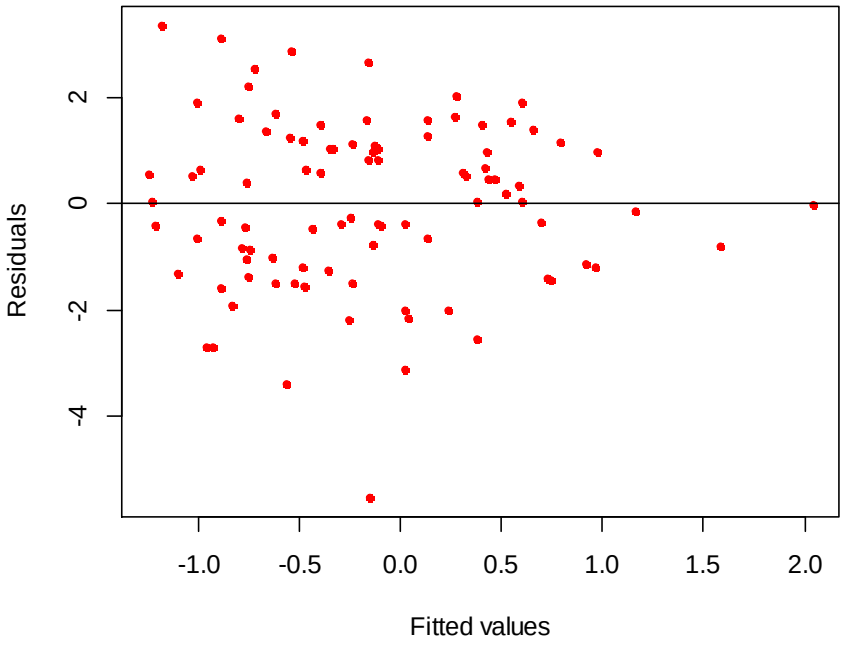
- **Media 0**

Para contrastar si los residuos tienen media 0, utilizamos el test t-Student.

One Sample t-test
data: fit_final$residuals
t = -3.2205e-16, df = 92, p-value = 1
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 -0.3229469 0.3229469
sample estimates:
mean of x
-5.236696e-17
El p-valor del test es 1 > 0.05 por lo que no puede rechazarse que los residuos tengan media 0.

- **Independencia**

Para comprobar que los residuos son independientes los representamos gráficamente frente a los valores ajustados.



No se observa ninguna tendencia en este gráfico, por tanto, se verifica la independencia de los residuos.

3) Modelos de regresión para el BPA e interacción con los factores de influencia.

3.1 Relation between bisphenols levels in urine.

Table SI-3. Spearman's correlation between bisphenols levels (ng·ml^{-1}) in urine.

	Bisphenol A	Bisphenol F	Bisphenol S
Bisphenol A	-	-0.082 (-0.271 – 0.113)	0.095 (-0.100 – 0.283)
Bisphenol F	-	-	-0.128 (-0.314 - 0.067)
Bisphenol S	-	-	-

3.2 Simple linear regression models to evaluate the relationship between bisphenols levels in urine and the other variables of the study.

Table SI-4. Relationship of bisphenol A levels in urine with the characteristics and habits of the study population.

Characteristics	Bisphenol A levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Mother				
Number of children				
1	2 (0.003 - 12)	-	-	-
2	0.8 (0.026 - 10)	-0.09420	(-0.836 - 0.648)	0.802
3 or more	0.6 (0.019 - 7)	-0.50800	(-1.73 - 0.718)	0.413
Age (years)	-	-0.00567	(-0.0835 - 0.0722)	0.885
Weight before pregnancy (kg)	-	0.0138	(-0.0195 - 0.0471)	0.414
Height (cm)	-	0.0386	(-0.0157 - 0.0929)	0.162
BMI before pregnancy (kg/m^2)	-	0.00836	(-0.081 - 0.0977)	0.853
Diet during pregnancy				
Yes	2 (0.133 - 8)	-	-	-
No	1.7 (0.003 - 12)	-0.435	(-1.44 - 0.571)	0.393
Country of birth				
Spain	1.7 (0.003 - 12)	-	-	-
Foreign	2.3 (0.063 - 10)	0.3790	(-0.627 - 1.39)	0.457
Place of residence				
Urban	1.9 (0.003 - 12)	-	-	-
Rural	2 (0.12 - 7.7)	-0.0661	(-0.981 - 0.848)	0.886
Education level				
Only primary school	1.2 (0.058 - 10.3)	-	-	-
Secundary school	1.9 (0.046 - 10)	0.60300	(-0.666 - 1.87)	0.348
University	1.5 (0.003 - 12)	-0.00463	(-1.11 - 1.1)	0.993
Occupational status				
Employed	1.9 (0.003 - 12)	-	-	-
Unemployed	0.6 (0.019 - 10)	-0.866	(-1.81 - 0.076)	0.071
Time worked outside the home (years)	-	-0.00804	(-0.0731 - 0.057)	0.807

Characteristics	Bisphenol A levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Use of cosmetics at work				
Yes	0.65 (0.133 - 7.3)	-	-	-
No	2 (0.003 - 12)	0.412	(-0.847 - 1.67)	0.517
Breastfed				
Yes	1 (0.003 - 12)	-	-	-
No	2 (0.026 - 10.3)	0.331	(-0.422 - 1.08)	0.386
Physical exercise				
3 or more days/week	0.5 (0.12 - 10.3)	-	-	-
1 or 2 days/week	2.3 (0.12 - 12)	0.6590	(-0.555 - 1.87)	0.284
Occasionally	2 (0.003 - 10)	-0.0425	(-1.07 - 0.982)	0.935
Never	0.65 (0.019 - 12)	-0.3020	(-1.43 - 0.826)	0.596
Smoker				
Yes	0.6 (0.083 - 10.3)	-	-	-
Ex-smoker	1.5 (0.026 - 12)	0.0249	(-1.38 - 1.43)	0.972
Never	2 (0.003 - 12)	0.2750	(-1.1 - 1.65)	0.693
Child				
Gestational age (weeks)	-	-0.122	(-0.441 - 0.196)	0.446
Sex				
Boy	0.95 (0.003 - 9.8)	-	-	-
Girl	2 (0.026 - 12)	0.359	(-0.34 - 1.06)	0.310
Weight (g)	-	-0.000081	(-0.000852 - 0.00069)	0.835
Height (cm)	-	0.0416	(-0.176 - 0.259)	0.703
Cranial perimeter (cm)	-	-0.0868	(-0.518 - 0.344)	0.686
Food consumption by groups (g/month)				
eggs	-	$5.46 \cdot 10^{-5}$	(-0.00107 - 0.00118)	0.924
Dairy products	-	$2.73 \cdot 10^{-5}$	($-2.03 \cdot 10^{-5}$ - $7.5 \cdot 10^{-5}$)	0.257
Meat products	-	$3.93 \cdot 10^{-5}$	(-0.000136 - 0.000215)	0.658

Characteristics	Bisphenol A levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Fishing products	-	0.000045	($-8.89\cdot10^{-5}$ - 0.000179)	0.506
Vegetables	-	$6.6\cdot10^{-6}$	($-4.15\cdot10^{-5}$ - $5.47\cdot10^{-5}$)	0.785
Fruits	-	$2.04\cdot10^{-5}$	($-9.23\cdot10^{-6}$ - $5\cdot10^{-5}$)	0.175
Legumes and cereals	-	0.000142	($1.83\cdot10^{-5}$ - 0.000265)	0.025*
Oils and fats	-	0.000597	(-0.000521 - 0.00171)	0.292
Pastries	-	$-1.72\cdot10^{-5}$	(-0.000294 - 0.00026)	0.902
Miscellany	-	$5.68\cdot10^{-5}$	(-0.000122 - 0.000236)	0.530
Drinks	-	$1.24\cdot10^{-5}$	($-2.06\cdot10^{-5}$ - $4.53\cdot10^{-5}$)	0.458
Packaged products (72h)	-	0.0391	(-0.00357 - 0.0819)	0.072
Cosmetics products				
Skin care				
Frecuency				
Never or before pregnancy	0.75 (0.088 - 12)	-	-	-
Daily	1 (0.019 - 9.8)	-0.0145	(-0.839 - 0.81)	0.972
Several times a week	2.4 (0.003 - 10)	0.5000	(-0.547 - 1.55)	0.346
Sometimes in the month	7.8 (2.4 - 12)	2.0700	(0.28 - 3.86)	0.024*
Parfums				
Frecuency				
Never or before pregnancy	0.65 (0.003 - 10)	-	-	-
Daily	2.35 (0.046 - 12)	0.853	(0.0606 - 1.65)	0.035*
Several times a week	1.6 (0.026 - 12)	0.458	(-0.428 - 1.35)	0.307
Sometimes in the month	4.45 (0.8 - 7.6)	1.630	(-0.109 - 3.36)	0.066
Deodorants				
Frecuency				
Never or before pregnancy	0.346 (0.026 - 2.4)	-	-	-
Daily	2 (0.003 - 12)	1.110	(-0.375 - 2.59)	0.141

Characteristics	Bisphenol A levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Several times a week	0.8 (0.185 - 2.6)	0.779	(-1.34 - 2.9)	0.468
Sun screen				
Frecuency				
Never or before pregnancy	1.9 (0.026 - 12)	-	-	-
Daily	0.6 (0.003 - 9.8)	-0.5120	(-1.5 - 0.474)	0.305
Several times a week	2.35 (0.088 - 10)	0.6360	(-0.532 - 1.8)	0.282
Sometimes in the month	0.492 (0.185 - 0.8)	-0.9750	(-3.44 - 1.49)	0.434
Ocasionally	2.6 (0.019 - 12)	-0.0312	(-1.25 - 1.19)	0.960
Hear colour				
Times/year	-	0.0124	(-0.0725 - 0.0973)	0.773
Last application				
≤1 week	2 (0.191 - 7)	-	-	-
< 1 month	1.2 (0.026 - 2.8)	-1.2000	(-2.9 - 0.509)	0.167
≥ 1 month, < 3 months	2.3 (0.019 - 12)	0.0446	(-1.26 - 1.35)	0.946
≥ 3 months	1.3 (0.058 - 9)	-0.5990	(-2.09 - 0.894)	0.428
Never or before pregnancy	0.7 (0.003 - 10)	-0.7980	(-2.03 - 0.438)	0.203
Lipstick				
Frecuency				
Never or before pregnancy	1.2 (0.003 - 12)	-	-	-
Daily	1.7 (0.171 - 4.2)	0.156	(-0.998 - 1.31)	0.789
Several times a week	3.7 (0.026 - 12)	0.800	(-0.554 - 2.15)	0.244
Sometimes in the month	1.9 (0.046 - 10.3)	0.534	(-0.572 - 1.64)	0.340
Makeup				
Frecuency				
Never or before pregnancy	1.2 (0.003 - 12)	-	-	-
Daily	1.9 (0.088 - 2.8)	0.0693	(-0.981 - 1.12)	0.896
Several times a week	3.1 (0.026 - 12)	0.4320	(-0.587 - 1.45)	0.402
Sometimes in the month	2.4 (0.046 - 9)	0.2800	(-0.664 - 1.22)	0.558

* P-value ≤ 0.05.

4) Modelos de regression par MP, EP y PP e interacción con los factores de influencia

4.1 Relation between parabens levels in urine.

Table SI-5. Spearman's correlation between parabens levels (ng·ml-1) in urine.

	Methyl paraben	Ethyl paraben	Propyl paraben	Butyl paraben
Methyl paraben	-	0.129 (-0.066 – 0.315)	-0.070 (-0.26 – 0.125)	0.013 (-0.181 – 0.206)
Ethyl paraben	-	-	0.333** (0.149 - 0.495)	-0.187 (-0.367 - 0.007)
Propyl paraben	-	-	-	-0.078 (-0.267 - 0.117)
Butyl paraben	-	-	-	-

** Correlation is significant at the 0.001 level.

4.2 Simple linear regression models to evaluate the relationship between parabens levels in urine and the other variables of the study.

Table SI-6. Relationship of methylparaben levels in urine with the characteristics and habits of the study population.

Characteristics	**Methylparaben levels Median (minimum - maximum)**	**Estimated parameters (Simple regression model)**	**CI (95 %)**	**P-value**
Mother				
Number of children				
1	15.6 (0.036 - 490)	-	-	-
2	60 (0.072 - 892)	0.865	(-0.25 - 1.98)	0.127
3 or more	150 (0.159 - 3052)	0.926	(-0.915 - 2.77)	0.321
Age (years)	-	-0.0882	(-0.205 - 0.029)	0.139
Weight before pregnancy (kg)	-	-0.0156	(-0.0663 - 0.035)	0.542
Height (cm)	-	-0.0475	(-0.13 - 0.0351)	0.257
BMI before pregnancy (kg/m²)	-	-0.0147	(-0.15 - 0.121)	0.830

Characteristics	Methylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Diet during pregnancy				
Yes	10 (2 - 587)	-	-	-
No	22 (0.036 - 3052)	-0.0823	(-1.61 - 1.45)	0.915
Country of birth				
Spain	22 (0.036 - 3052)	-	-	-
Foreign	15.6 (0.158 - 630)	0.497	(-1.03 - 2.03)	0.52
Place of residence				
Urban	15.4 (0.036 - 3052)	-	-	-
Rural	74.6 (0.072 - 350)	0.117	(-1.27 - 1.51)	0.867
Education level				
Only primary school	60 (0.159 - 630)	-	-	-
Secundary school	26 (0.072 - 587)	-0.0298	(-1.97 - 1.91)	0.976
University	15.6 (0.036 - 3052)	-0.3310	(-2.02 - 1.36)	0.699
Occupational status				
Employed	24.65 (0.036 - 3052)	-	-	-
Unemployed	20 (0.159 - 560)	0.0649	(-1.39 - 1.52)	0.93
Time worked outside the home (years)	-	0.0262	(-0.0717 - 0.124)	0.596
Use of cosmetics at work				
Yes	108.1 (4 - 587)	-	-	-
No	16 (0.036 - 3052)	-1.46	(-3.35 - 0.433)	0.129
Breastfed				
Yes	13.75 (0.072 - 3052)	-	-	-
No	26 (0.036 - 452)	0.204	(-0.954 - 1.36)	0.727
Physical exercise				
3 or more days/week	6.3 (0.158 - 268)	-	-	-
1 or 2 days/week	29.2 (0.127 - 490)	1.210	(-0.644 - 3.07)	0.198
Occasionally	15.7 (0.036 - 3052)	0.792	(-0.776 - 2.36)	0.319
Never	41 (0.072 - 560)	0.689	(-1.04 - 2.42)	0.430
Smoker				

Characteristics	Methylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Yes	10.5 (0.108 - 135.4)	-	-	-
Ex-smoker	65 (0.036 - 892)	1.56	(-0.55 - 3.67)	0.146
Never	10.4 (0.036 - 3052)	0.90	(-1.17 - 2.97)	0.390
Child				
Gestational age (weeks)	-	0.385	(-0.0708 - 0.842)	0.097
Sex				
Boy	11.2 (0.036 - 3052)	-	-	-
Girl	27.25 (0.106 - 892)	0.666	(-0.383 - 1.71)	0.211
Weight (g)	-	0.00113	($-3.35\cdot10^{-6}$ - 0.00227)	0.051
Height (cm)	-	0.313	(0.00781 - 0.619)	0.045*
Cranial perimeter (cm)	-	-0.117	(-0.758 - 0.524)	0.714
Food consumption by groups (g/month)				
eggs	-	0.000398	(-0.00133 - 0.00212)	0.648
Dairy products	-	$3.11\cdot10^{-5}$	($-4.2\cdot10^{-5}$ - 0.000104)	0.401
Meat products	-	0.000122	(-0.000145 - 0.00039)	0.366
Fishing products	-	0.000241	($4.14\cdot10^{-5}$- 0.000441)	0.019*
Vegetables	-	$1.03\cdot10^{-5}$	($-6.34\cdot10^{-5}$- $8.39\cdot10^{-5}$)	0.783
Fruits	-	$-1.4\cdot10^{-6}$	($-4.72\cdot10^{-5}$- $4.44\cdot10^{-5}$)	0.953
Legumes and cereals	-	0.000128	($-6.46\cdot10^{-5}$- 0.00032)	0.191
Oils and fats	-	$6.71\cdot10^{-5}$	(-0.00165 - 0.00179)	0.939
Pastries	-	0.00034	(-7.78e-05 - 0.000758)	0.109
Miscellany	-	0.000119	(-0.000155 - 0.000392)	0.391
Drinks	-	$-1.44\cdot10^{-5}$	($-6.48\cdot10^{-5}$- $3.61\cdot10^{-5}$)	0.573

Characteristics	Methylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Packaged products (72h)	-	0.0721	(0.0078 - 0.136)	0.028*
Cosmetics products				
Skin care				
Frecuency				
Never or before pregnancy	37.15 (0.036 - 354)	-	-	-
Daily	26.6 (0.036 - 3052)	0.627	(-0.667 - 1.92)	0.339
Several times a week	18 (0.4 - 258)	-0.190	(-1.83 - 1.45)	0.819
Sometimes in the month	5 (0.158 - 15)	-1.700	(-4.51 - 1.11)	0.234
Parfums				
Frecuency				
Never or before pregnancy	6.15 (0.036 - 560)	-	-	-
Daily	43 (0.072 - 3052)	1.320	(0.116 - 2.52)	0.032*
Several times a week	86.2 (0.127 - 892)	1.640	(0.296 - 2.99)	0.017*
Sometimes in the month	13 (0.158 - 243)	-0.259	(-2.89 - 2.37)	0.846
Deodorants				
Frecuency				
Never or before pregnancy	9.25 (1.5 - 892)	-	-	-
Daily	24 (0.036 - 3052)	0.301	(-1.87 - 2.48)	0.784
Several times a week	68 (1 - 90.2)	0.230	(-2.89 - 3.35)	0.884
Sun screen				
Frecuency				
Never or before pregnancy	27.6 (0.036 - 3052)	-	-	-
Daily	15.4 (0.108 - 452)	-0.4250	(-1.92 - 1.07)	0.575
Several times a week	23 (0.158 - 490)	-0.2470	(-2.02 - 1.53)	0.783
Sometimes in the month	52 (24 - 80)	0.7410	(-3 - 4.49)	0.695
Ocasionally	15 (1 - 560)	-0.0947	(-1.95 - 1.76)	0.920
Hear colour				
Times/year	-	-0.0803	(-0.207 - 0.0468)	0.213
Last application				
≤1 week	5 (1 - 452)	-	-	-

Characteristics	Methylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
< 1 month	15.4 (0.072 - 3052)	-0.1980	(-2.86 - 2.46)	0.883
≥ 1 month, < 3 months	15 (0.036 - 560)	-0.3020	(-2.33 - 1.73)	0.769
≥ 3 months	42.55 (0.127 - 368)	0.0622	(-2.26 - 2.39)	0.958
Never or before pregnancy	26 (0.036 - 630)	0.1280	(-1.8 - 2.05)	0.895
Lipstick				
Frecuency				
Never or before pregnancy	20 (0.036 - 587)	-	-	-
Daily	79.9 (0.8 - 3052)	0.816	(-0.936 - 2.57)	0.358
Several times a week	29.2 (0.036 - 892)	0.297	(-1.76 - 2.35)	0.775
Sometimes in the month	93 (1.5 - 490)	0.994	(-0.686 - 2.67)	0.243
Makeup				
Frecuency				
Never or before pregnancy	15.4 (0.036 - 560)	-	-	-
Daily	24 (0.8 - 3052)	0.588	(-0.987 - 2.16)	0.460
Several times a week	68 (0.106 - 892)	0.557	(-0.971 - 2.09)	0.471
Sometimes in the month	26 (1.5 - 630)	1.230	(-0.185 - 2.65)	0.088

* P-value ≤ 0.05.

Table SI-7. Relationship of ethylparaben levels in orine with the characteristics and habits of the study population.

Characteristics	Ethylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Mother				
Number of children				
1	0.9 (0.014 - 234)	-	-	-
2	0.9 (0.006 - 20.5)	-0.588	(-1.51 - 0.336)	0.210
3 or more	0.5 (0.015 - 7.6)	-0.455	(-1.98 - 1.07)	0.556
Age (years)	-	-0.0397	(-0.137 - 0.0579)	0.422

Characteristics	Ethylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Weight before pregnancy (kg)	-	-0.0893	(-0.128 - -0.0506)	< 0.001*
Height (cm)	-	-0.00829	(-0.0782 - 0.0616)	0.814
BMI before pregnancy (kg/m²)	-	-0.235	(-0.339 - -0.131)	< 0.001*
Diet during pregnancy				
Yes	0.5 (0.006 - 20.5)	-	-	-
No	0.95 (0.012 - 234)	0.377	(-0.907 - 1.66)	0.561
Country of birth				
Spain	0.9 (0.012 - 234)	-	-	-
Foreign	0.9 (0.006 - 20.5)	-1.0700	(-2.34 - 0.199)	0.098
Place of residence				
Urban	0.9 (0.006 - 166)	-	-	-
Rural	2.6 (0.024 - 234)	0.834	(-0.332 - 2)	0.159
Education level				
Only primary school	0.9 (0.014 - 7.5)	-	-	-
Secundary school	0.6 (0.006 - 32)	-0.012	(-1.61 - 1.59)	0.988
University	0.9 (0.012 - 234)	0.361	(-1.03 - 1.76)	0.608
Occupational status				
Employed	0.85 (0.006 - 234)	-	-	-
Unemployed	1.2 (0.012 - 72.5)	-0.356	(-1.55 - 0.842)	0.557
Time worked outside the home (years)	-	-0.0519	(-0.132 - 0.0286)	0.204
Use of cosmetics at work				
Yes	5.35 (0.083 - 32)	-	-	-
No	0.8 (0.006 - 234)	-1.44	(-2.99 - 0.116)	0.069
Breastfed				
Yes	0.9 (0.006 - 234)	-	-	-
No	1 (0.012 - 59.3)	-0.155	(-1.09 - 0.785)	0.744
Physical exercise				
3 or more days/week	2 (0.006 - 234)	-	-	-

Characteristics	Ethylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
1 or 2 days/week	0.5 (0.024 - 32)	-1.190	(-2.66 - 0.281)	0.112
Occasionally	0.95 (0.012 - 72.5)	-0.914	(-2.16 - 0.327)	0.147
Never	0.25 (0.015 - 20.5)	-1.660	(-3.03 - -0.293)	0.018*
Smoker				
Yes	0.5 (0.19 - 166)	-	-	-
Ex-smoker	0.5 (0.012 - 234)	-0.719	(-2.48 - 1.04)	0.419
Never	1 (0.006 - 72.5)	-0.727	(-2.45 - 0.998)	0.405
Child				
Gestational age (weeks)	-	-0.145	(-0.535 - 0.245)	0.462
Sex				
Boy	0.75 (0.012 - 234)	-	-	-
Girl	1.1 (0.006 - 166)	0.260	(-0.63 - 1.15)	0.563
Weight (g)	-	$-5.34 \cdot 10^{-5}$	(-0.00103 - 0.000925)	0.914
Height (cm)	-	0.00274	(-0.267 - 0.272)	0.984
Cranial perimeter (cm)	-	0.00862	(-0.594 - 0.611)	0.977
Food consumption by groups (g/month)				
eggs	-	0.00121	(-0.000179 - 0.0026)	0.087
Dairy products	-	$1.64 \cdot 10^{-5}$	($-4.36 \cdot 10^{-5}$ - 7.64e-05)	0.589
Meat products	-	$4.14 \cdot 10^{-5}$	(-0.000179 - 0.000261)	0.710
Fishing products	-	$-1.60 \cdot 10^{-6}$	(-0.00017 - 0.000167)	0.985
Vegetables	-	$-5.24 \cdot 10^{-5}$	($-0.000112 - 7 \cdot 10^{-6}$)	0.083
Fruits	-	$-2.46 \cdot 10^{-5}$	($-6.17 \cdot 10^{-5}$ - $1.25 \cdot 10^{-5}$)	0.192
Legumes and cereals	-	0.000123	($-3.4 \cdot 10^{-5}$ - 0.00028)	0.123
Oils and fats	-	-0.000083	(-0.00149 - 0.00133)	0.907
Pastries	-	$-8.68 \cdot 10^{-5}$	(-0.000433 - 0.00026)	0.620

Characteristics	Ethylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Miscellany	-	$-3.07 \cdot 10^{-5}$	(-0.000255 - 0.000194)	0.787
Drinks	-	$-1.82 \cdot 10^{-5}$	($-5.93 \cdot 10^{-5}$ - $2.3 \cdot 10^{-5}$)	0.384
Packaged products (72h)	-	0.0195	(-0.0348 - 0.0737)	0.478
Cosmetics products				
Skin care				
Frecuency				
Never or before pregnancy	0.75 (0.045 - 166)	-	-	-
Daily	0.95 (0.012 - 234)	0.240	(-0.842 - 1.32)	0.661
Several times a week	0.7 (0.006 - 18.7)	-0.265	(-1.64 - 1.11)	0.702
Sometimes in the month	0.208 (0.058 - 3)	-0.975	(-3.32 - 1.37)	0.412
Parfums				
Frecuency				
Never or before pregnancy	0.95 (0.006 - 166)	-	-	-
Daily	1 (0.012 - 32)	-0.1810	(-1.21 - 0.843)	0.726
Several times a week	0.35 (0.012 - 234)	-0.6480	(-1.79 - 0.499)	0.265
Sometimes in the month	1 (0.3 - 1.9)	-0.0668	(-2.31 - 2.18)	0.953
Deodorants				
Frecuency				
Never or before pregnancy	0.785 (0.023 - 2.9)	-	-	-
Daily	0.9 (0.006 - 234)	0.705	(-1.15 - 2.56)	0.452
Several times a week	1.3 (0.173 - 20.5)	1.070	(-1.59 - 3.73)	0.425
Sun screen				
Frecuency				
Never or before pregnancy	0.95 (0.006 - 166)	-	-	-
Daily	0.8 (0.012 - 59.3)	-0.035	(-1.25 - 1.18)	0.955
Several times a week	2.65 (0.076 - 234)	1.320	(-0.115 - 2.76)	0.071
Sometimes in the month	11.2 (1.9 - 20.5)	2.120	(-0.911 - 5.15)	0.168
Ocasionally	0.2 (0.015 - 72.5)	-0.820	(-2.33 - 0.685)	0.282
Hear colour				

Characteristics	Ethylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Times/year		-0.0265	(-0.13 - 0.0765)	0.610
Last application				
≤1 week	2 (0.17 - 59.3)	-	-	-
< 1 month	0.136 (0.012 - 6)	-1.660	(-3.85 - 0.524)	0.134
≥ 1 month, < 3 months	0.7 (0.015 - 72.5)	-0.664	(-2.33 - 1.01)	0.432
≥ 3 months	0.4 (0.067 - 234)	-0.595	(-2.51 - 1.32)	0.538
Never or before pregnancy	0.9 (0.006 - 166)	-0.731	(-2.32 - 0.853)	0.362
Lipstick				
Frecuency				
Never or before pregnancy	0.8 (0.012 - 234)	-	-	-
Daily	1.55 (0.017 - 5.7)	0.250	(-1.2 - 1.7)	0.733
Several times a week	0.4 (0.012 - 14)	-0.726	(-2.43 - 0.977)	0.400
Sometimes in the month	1.2 (0.006 - 72.5)	0.445	(-0.946 - 1.84)	0.527
Makeup				
Frecuency				
Never or before pregnancy	0.5 (0.006 - 166)	-	-	-
Daily	1.4 (0.017 - 14)	0.725	(-0.602 - 2.05)	0.281
Several times a week	0.65 (0.012 - 234)	0.534	(-0.754 - 1.82)	0.413
Sometimes in the month	1.5 (0.014 - 72.5)	0.762	(-0.431 - 1.96)	0.208

* P-value ≤ 0.05.

Table SI-8. Relationship of prophylparaben levels in urine with the characteristics and habits of the study population.

Characteristics	Prophylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Mother				
Number of children				
1	0.172 (0 - 230)	-	-	-

Characteristics	Prophylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
2	0.188 (0.002 - 54)	0.149	(-1.06 - 1.36)	0.807
3 or more	0.5 (0.065 - 8)	0.910	(-1.09 - 2.9)	0.368
Age (years)	-	0.0871	(-0.0392 - 0.213)	0.174
Weight before pregnancy (kg)	-	-0.0607	(-0.115 - -0.00653)	0.028*
Height (cm)	-	-0.0113	(-0.102 - 0.0796)	0.806
BMI before pregnancy (kg/m²)	-	-0.156	(-0.301 - -0.0107)	0.036*
Diet during pregnancy				
Yes	0.172 (0.001 - 20)	-	-	-
No	0.196 (0 - 230)	0.689	(-0.979 - 2.36)	0.414
Country of birth				
Spain	0.2 (0 - 230)	-	-	-
Foreign	0.091 (0.007 - 8)	-0.346	(-2.02 - 1.33)	0.682
Place of residence				
Urban	0.126 (0.001 - 230)	-	-	-
Rural	0.197 (0 - 11.2)	0.426	(-1.13 - 1.98)	0.589
Education level				
Only primary school	0.109 (0.001 - 7.5)	-	-	-
Secundary school	0.126 (0 - 230)	0.954	(-1.12 - 3.03)	0.363
University	0.3 (0.002 - 54)	1.140	(-0.659 - 2.95)	0.211
Occupational status				
Employed	0.196 (0 - 230)	-	-	-
Unemployed	0.112 (0.003 - 33.5)	0.286	(-1.27 - 1.84)	0.716
Time worked outside the home (years)	-	-0.0539	(-0.158 - 0.0503)	0.307
Use of cosmetics at work				
Yes	0.205 (0.001 - 230)	-	-	-
No	0.196 (0 - 54)	0.622	(-1.43 - 2.67)	0.549
Breastfed				
Yes	0.25 (0 - 230)	-	-	-
No	0.091 (0.002 - 54)	-0.725	(-1.94 - 0.488)	0.238

Characteristics	Prophylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Physical exercise				
3 or more days/week	0.09 (0.002 - 20)	-	-	-
1 or 2 days/week	0.5 (0.008 - 230)	1.170	(-0.771 - 3.11)	0.234
Occasionally	0.194 (0 - 54)	-0.504	(-2.14 - 1.14)	0.544
Never	0.177 (0.002 - 54)	-0.408	(-2.21 - 1.4)	0.654
Smoker				
Yes	0.065 (0.015 - 20)	-	-	-
Ex-smoker	0.2 (0 - 230)	0.485	(-1.81 - 2.78)	0.675
Never	0.196 (0.002 - 54)	0.497	(-1.75 - 2.75)	0.662
Child				
Gestational age (weeks)	-	-0.365	(-0.87 - 0.139)	0.153
Sex				
Boy	0.35 (0.008 - 230)	-	-	-
Girl	0.118 (0 - 54)	-1.040	(-2.19 - 0.104)	0.074
Weight (g)	-	0.00109	(-0.000166 - 0.00235)	0.088
Height (cm)	-	0.173	(-0.182 - 0.529)	0.334
Cranial perimeter (cm)	-	0.127	(-0.621 - 0.874)	0.733
Food consumption by groups (g/month)				
eggs	-	$7.54 \cdot 10^{-5}$	(-0.00175 - 0.0019)	0.935
Dairy products	-	$-3.54 \cdot 10^{-5}$	(-0.000113 - $4.18 \cdot 10^{-5}$)	0.365
Meat products	-	0.000134	(-0.000148 - 0.000417)	0.349
Fishing products	-	-0.000166	(-0.00038 - $4.87 \cdot 10^{-5}$)	0.128
Vegetables	-	$-2.12 \cdot 10^{-5}$	($-9.89 \cdot 10^{-5}$ - $5.65 \cdot 10^{-5}$)	0.589
Fruits	-	$-3.57 \cdot 10^{-5}$	($-8.34 \cdot 10^{-5}$- $1.21 \cdot 10^{-5}$)	0.142
Legumes and cereals	-	$4.68 \cdot 10^{-5}$	(-0.000158 - 0.000251)	0.651

Characteristics	Prophylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Oils and fats	-	0.000986	(-0.00082 - 0.00279)	0.281
Pastries	-	-0.000463	($-9\cdot10^{-4}$ - $-2.59\cdot10^{-5}$)	0.038*
Miscellany	-	-0.000146	(-0.000434 - 0.000143)	0.318
Drinks	-	$-6.10\cdot10^{-6}$	($-5.94\cdot10^{-5}$- $4.72\cdot10^{-5}$)	0.821
Packaged products (72h)	-	-0.00377	(-0.0744 - 0.0669)	0.916
Cosmetics products				
Skin care				
Frecuency				
Never or before pregnancy	0.033 (0 - 7)	-	-	-
Daily	0.3 (0.001 - 230)	1.94	(0.591 - 3.3)	0.005*
Several times a week	0.5 (0.004 - 54)	1.99	(0.269 - 3.71)	0.024*
Sometimes in the month	4.521 (0.01 - 54)	2.73	(-0.206 - 5.67)	0.068
Parfums				
Frecuency				
Never or before pregnancy	0.165 (0 - 54)	-	-	-
Daily	0.45 (0.002 - 230)	0.994	(-0.33 - 2.32)	0.139
Several times a week	0.09 (0.004 - 54)	-0.220	(-1.7 - 1.26)	0.768
Sometimes in the month	0.121 (0.003 - 1.8)	-0.719	(-3.62 - 2.18)	0.623
Deodorants				
Frecuency				
Never or before pregnancy	0.035 (0.009 - 7)	-	-	-
Daily	0.3 (0 - 230)	1.1200	(-1.25 - 3.49)	0.350
Several times a week	0.196 (0.002 - 0.9)	0.0482	(-3.34 - 3.44)	0.978
Sun screen				
Frecuency				
Never or before pregnancy	0.198 (0 - 54)	-	-	-
Daily	0.158 (0.002 - 4.7)	-0.596	(-2.19 - 0.998)	0.460
Several times a week	2.9 (0.021 - 230)	1.790	(-0.0972 - 3.68)	0.063

Characteristics	Prophylparaben levels Median (minimum - maximum)	Estimated parameters (Simple regression model)	CI (95 %)	P-value
Sometimes in the month	0.025 (0.003 - 0.047)	-2.880	(-6.86 - 1.1)	0.154
Ocasionally	0.4 (0.008 - 54)	0.654	(-1.32 - 2.63)	0.513
Hear colour				
Times/year	-	0.0748	(-0.0634 - 0.213)	0.285
Last application				
≤1 week	3 (0.003 - 10)	-	-	-
< 1 month	0.197 (0.014 - 11.2)	-1.770	(-4.56 - 1.02)	0.210
≥ 1 month, < 3 months	0.126 (0.002 - 230)	-1.220	(-3.34 - 0.912)	0.260
≥ 3 months	0.344 (0.033 - 6)	-0.742	(-3.18 - 1.7)	0.547
Never or before pregnancy	0.158 (0 - 20)	-1.960	(-3.98 - 0.0594)	0.057
Lipstick				
Frecuency				
Never or before pregnancy	0.176 (0 - 54)	-	-	-
Daily	0.3 (0.007 - 10)	0.943	(-0.904 - 2.79)	0.314
Several times a week	0.031 (0.008 - 2)	-0.860	(-3.03 - 1.31)	0.433
Sometimes in the month	1.8 (0.003 - 230)	1.760	(-0.0139 - 3.53)	0.052
Makeup				
Frecuency				
Never or before pregnancy	0.176 (0.001 - 54)	-	-	-
Daily	0.2 (0.003 - 39.3)	0.378	(-1.36 - 2.12)	0.668
Several times a week	0.196 (0.008 - 7.5)	0.188	(-1.5 - 1.88)	0.825
Sometimes in the month	0.5 (0 - 230)	0.787	(-0.778 - 2.35)	0.321

* P-value